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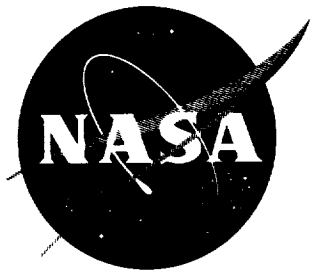

TECHNICAL NOTE

D-1357

PROCEEDINGS OF CONFERENCE ON SPACECRAFT STERILIZATION

Held Under the Auspices of the
NASA Biosciences Programs
July 9, 1962

Edited by Freeman H. Quimby

NASA Headquarters
Washington, D. C.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

WASHINGTON December 1962

FOREWORD

In the interest of producing a useful and relatively prompt record of the NASA Sterilization Conference, the editor took numerous shortcuts, including first of all, the decision not to mail the stenotypist's draft to the conferees for correction and return. The second major decision was to eliminate from the record the discussion of the proposed guidelines, but merely to include the guidelines as finally modified by the conference. The remainder of the deliberations, largely those of the morning session are essentially verbatim except for occasional unproductive exchanges, obvious mistakes in transcription and the perpetual corrections in the use of 10^{-4} and 10^{4}. There are instances when the editor rephrased statements to more succinctly state what he thought the participant was trying to "get over" to the group. Many garbled or incomplete sentences in the draft were eliminated. Detailed editing was not done, such as sentence structure, grammar, or even numbers and figures which the editor believes incorrect but nevertheless appeared in the transcript. The recommendations for R&D received from the participants by mail are recorded here, with the hope that none were accidentally left out.

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SUMMARY

The proceedings of a conference on spacecraft sterilization are presented. The conference took place July 9, 1962, in Washington, D.C., under the auspices of the NASA Bioscience Programs. The information presented is essentially a transcript of the discussions, with editing kept to a minimum in the interest of preserving the individual contributions of the participants. Recommendations of the participants for research and development are appended.

CONFERENCE PROCEEDINGS

The meeting was called to order at 9:00 a.m. on Monday, July 9, 1962. Those present were:

Dr. Orr Reynolds, NASA Headquarters, Co-Chairman

Dr. George Hobby, JPL, Co-Chairman

Dr. Freeman H. Quimby, NASA headquarters

Mr. Rolf Hastrup, JPL

Dr. John B. Opfell, Dynamic Science Corporation

Dr. Norman Horowitz, California Institute of Technology

Dr. Arthur Cherkin, Don Baxter, Inc.

Dr. Charles Phillips, Ft. Detrick

Mr. Briggs Phillips, Ft. Detrick

Dr. Carl Bruch, Schwarz Laboratories

Dr. Daniel Tompkins, Aeronutronics

Dr. William Oswald, University of California

Dr. Richard Price, General Electric Co.

Mr. Harold Wolf, Calif. Institute of Technology

Dr. John Perkins, American Sterilizer Co.

Mr. Robert Varga, Hughes Aircraft Co.

Dr. Victoria Lynch, Lockheed

Mr. Albin Nowitsky, Lockheed

Dr. E. Staten Wynne, School of Aerospace Medicine, USAF

Mr. Leonard R. Piasecki, JPL

DR. QUIMBY: I might make a few opening comments. There have been a number of biological groups in the past who have met and deliberated the spacecraft sterilization problem. These have been generally referred to as CETEX, WESTEX, and the Armed Forces Bio-Astronautics Committee. However, these groups were restricted almost entirely to biologists and to biological considerations and to the matter of setting the standards that we should try to achieve in the sterility of spacecraft.

There has been much concern in the meantime over the fact that sterilization procedures represent a hazard to the success of the total mission. This is particularly true at headquarters. I don't know about JPL. But it occurred to Dr. Reynolds, who suggested this meeting, that we might have a profitable conference if we were to bring in a unique group of people, namely, biologists, sanitary engineers, and spacecraft engineers, and approach it from the standpoint of not what should we do, but what <u>can</u> we do, and <u>how</u> should we do it.

DR. REYNOLDS: I gather that everybody doesn't have an agenda, therefore I will read the objectives that are given as the first item on the agenda. The first objective, to review the current spacecraft sterilization procedures and to generate recommendations for their improvement. That is one of the objectives of this conference.

Second: To obtain recommendations on areas of research and development in the spacecraft sterilization program.

The second item on our agenda is the status of current sterilization efforts. I would like to call on Dr. Hobby to lead the discussion

on this item.

DR. HOBBY: Some of you are familiar with, and actually have participated in, the current sterilization effort. However, I realize that some of you have not been in the field and do not have the background that would be helpful for your participation today. Therefore, I have asked Dr. Rolf Hastrup of the Jet Propulsion Lab, to give us a brief outline of the current status of our sterilization program.

MR. HASTRUP: For those of you who are not familiar with the effort that has been going on with the lunar program, I will just briefly go over the procedures which are currently being employed.

There are two basic objectives in the present effort to apply sterilization procedures to lunar spacecraft.

The first of these is the straightforward one of limiting possible contamination of the moon by viable organisms.

The second objective is to advance the state-of-the-art and contribute to the development of more effective and reliable methods which could be applied to the sterilization of spacecraft. This is critical if we are to successfully accomplish sterilization of future planetary spacecraft or spacecraft capsules.

It is not possible to utilize directly the procedures found in the literature and applied currently in the food industry or in medicine. Procedures must be developed further to accomplish complete sterilization of all viable organisms anywhere on or within the components of the spacecraft. Of course these procedures must also be compatible with the complex spacecraft design as well as the great number of tests and operations the spacecraft goes through before launch and ascent through the atmosphere.

With these objectives, sterilization requirements have been imposed on both Ranger and Surveyor spacecraft.

On Ranger the approach is to accomplish sterilization in three general phases. First, all subassemblies are required to be sterilized internally. This is accomplished in nearly every case by heating the subassemblies to 125° Centigrade for 24 hours after thermal equilibrium has been reached. An alternative cycle is 160° Centigrade for two hours. These cycles were first established for lunar spacecraft about two years ago on the basis of available information in the literature on dry heat sterilization as well as a few supplemental tests which were run on resistance spores at that time. Although excellent progress has been made in the design of spacecraft for heat sterilization, a few critical items cannot tolerate the required temperature cycle. Where possible, these critical components have been handled by other techniques involving chemical agents during sterile manufacture. For example, additives have been used in plastic resin systems to render them self-sterilizing when formed. The thermal cycle is preferred, however, because the alternative chemical methods are not considered as reliable from the sterilization standpoint.

DR. HOBBY: Rolf, I wonder if I may interrupt for a moment to comment in a little more detail on those components which could not be subjected to the heat sterilization cycle. Particularly those which were not sterilizable even by additives or by sterile assembly techniques. Probably greater than 90-95% of the total number were subjected to the thermal cycle. However, even though the actual number that could not be heat-sterilized was small, some of the major components had to be included

in this category. For example, the 200 lb. retromotor for the Ranger 3, 4, 5 capsule could not be heat sterilized. Some preliminary tests which were performed at Dr. Carl Bruch's laboratory indicate a contamination level for solid propellants of about 50 organisms per gm. This would indicate a total population of around 10^6 organisms. However, the energy of impact of retromotor would probably cause it to burn if it did not properly ignite. Other non-heatable components which we consider to contribute an insignificant amount to the internal contamination levels are batteries, pyrotechnic switches, photomultiplier tubes, and germanium transistors.

MR. HASTRUP: On Ranger, assurance of internal sterilization of subassemblies has been the responsibility of the cognizant engineers. That is, of each subassembly it has been the requirement placed on the cognizant engineer to deliver that in an internally sterilized fashion. It has been up to them to determine just how to do it, in most cases they having heat sterilized the subassembly.

The second phase in Ranger sterilization operations is the assembly of the internally sterilized subassembly packages into a complete spacecraft. At present, this is an impossible task if 100 percent sterilization of the complete spacecraft is desired. The spacecraft consists of thousands of individual parts and even when combined as sub-assemblies, hundreds of items are involved. Furthermore it is not merely a matter of a simple assembly operation. Typically, assembly operations are begun on a Ranger spacecraft eight or nine months before launch. After initial assembly the spacecraft is put through many tests under different environmental conditions. All through the course of these operations the

spacecraft is probed, changed in configuration, actuated, torn down repeatedly for repair, modification or recalibration and transported around from one test facility to another, including the trip from JPL to the Cape. Some of these operations would not be repeated unnecessarily, but many cannot, and there is always a conflicting desire to run a final test after any operation such as sterile assembly. We have been developing new techniques using liquid and grease sterilants to avoid the necessity of time-consuming and cumbersome sterile glovebox operations. These sterilants may not be completely satisfactory as yet, and their development has actually come too late to permit utilization in the Ranger project because they were not available in time to be included in the type of approval testing of hardware to which they would have been applied.

There are also severe problems in toxicity in handling some of the sterilants. So, in most of our field assembly operations on Ranger, we have been limited to the practice of careful cleaning procedures and/or swabbing of mating surfaces with alcohol disinfectant. Sterile glove box operations with the use of ETO are being carried out and some subassemblies such as the seismometer capsule. Membrane and fibrous type filters are also used during assembly and test phase for sterilizing liquids and gases which must be supplied to the spacecraft. The pore size of the membrane filters is either 0.22 or 0.45 micron. The fibrous filters have been tested and found to have a penetration of about one in 10^6 of B subtilis niger spores. The final phase of Ranger sterilization is under much better control.

Shortly before launch the completed spacecraft assembly in its final configuration is enclosed within the nose-cone shroud and biologically sealed from further outside contamination. An ethylene oxide gas mixture

containing about 450 milligrams per liter of ETO is introduced into the nose cone cavity and held there for 11 hours under slight positive pressure and at from 30 to 50 percent relative humidity. The sterilant vapor is then purged away with nitrogen gas which has been sterilized by filtration. This operation is conducted in a special explosive-safe facility near the launch stand. From there the spacecraft is transported in its sterile sealed condition to the launch stand where it is mated to the launch vehicle. The bacteriological seal is maintained throughout all these operations and until the shroud is ejected at an altitude in excess of 300,000 feet, above which no bacteria are believed to exist.

The task on Surveyor is at least as great because the spacecraft is larger and even more complex than Ranger. Essentially, the same procedures will be used for Surveyor; however, the Surveyor schedule permits much of the experience and information gained on Ranger to be used to advantage. On Surveyor, greater emphasis is being put on heat sterilization of the largest possible subassembly in order to minimize sterile assembly operations. Furthermore, the additional time available provides a better opportunity to qualify liquids and grease sterilants for use in field assembly operations.

The sterilization constraint on the lunar missions has been given a priority below that of reliability or mission schedule. On that basis a few sterilization waivers have been allowed for critical components which are not compatible with existing procedures. Also, field assembly procedures have been permitted which cannot be considered completely effective. Nevertheless, considerable progress has been made in the sterilization of lunar spacecraft with the result that contamination has been kept to a very low level and much useful experience and information has been obtained which

can be utilized in establishing planetary requirements and procedures. However, many of those who are responsible for and working closest to the Ranger and Surveyor projects believe that the sterilization requirements do seriously reduce reliability in spite of the relative priorities which have been assigned. It is also expensive in terms of manpower and money. Therefore, it is important to carefully review the lunar sterilization requirements to determine that they are still justified or to what extent they may possibly be relaxed.

I think most of you have had a chance to look at Dr. Jaffe's report, which I think was sent out to the attendees. If we can concur with his conclusion that sterilization of lunar spacecraft should not be necessary for the sake of limiting contamination on the moon, since he believes that normal, clean assembly and operations would limit the contamination to a satisfactory low level, or if we would only gas sterilize the exterior portion of the spacecraft, then we should seriously consider the question of internal sterilization on the lunar spacecraft to see if it could possibly be relaxed.

For example, on the lunar spacecraft I have mentioned that the heat sterilization cycle is 125 degrees Centigrade for 24 hours. The hardware is actually type-approval tested at 125 degrees for 36 hours. The proposed planetary requirement is 135 degrees for 24 hours, and furthermore, it is recommended that the hardware be type-approval tested at a higher temperature than that, at 145 degrees Centigrade for 36 hours, and for three cycles. There are several reasons for this. One is to allow for tolerance in the ovens, which may be two or three degrees. Another is to provide statistical reliability where we test only a few items. We should provide

some margin for statistical variations in the hardware. And a third is that we should allow for overheating of some items in order to permit the heat to get to the most thermally isolated item in the complete spacecraft.

If the planetary requirement is to be established at a higher level than that which is currently being used in the lunar program, then at this point there may be a serious question as to how much further information we will be able to utilize from the lunar program.

We, of course, have been able to determine many critical items, weak points, and gain much practical experience in interior sterilization of lunar spacecraft. But I would think that at this point, possibly, we should concentrate our attention directly to the planetary spacecraft because it is costing us a great deal in the lunar program in terms of money and reliability, and we may get more from this effort if we apply it directly to the planetary program now that the planetary program is coming into shape, since we have established, at least tentatively, the requirements for the planetary program as well as begun design on the planetary spacecraft.

DR. HOBBY: Would anyone like to ask any questions?

DR. CHERKIN: How much is it costing in time and money in percentage of the total?

MR. HASTRUP: This is a very important question. I myself don't know of any very useful information which can be used in answer.

George, do you have any idea how we could express the cost in terms of dollars?

DR. HOBBY: It is a very difficult question. We can add up the

number of manhours and the costs of our contracts and of our in-house programs. On the other hand, the total expenditure might also be measured in terms of what it does to the mission reliability. If you try to fly a spacecraft and the reliability is reduced by the sterilization requirement and you have a mission failure, then of course the cost becomes considerably higher than if you just base the cost in terms of manpower and dollars.

It is not an easy number to arrive at. I wouldn't attempt to try it at the moment.

DR. HOROWITZ: Do the engineers think that the ethylene oxide treatment is reducing the reliability of payloads?

MR. HASTRUP: We haven't found many signs of compatibility problems with ethylene oxide. I think in general it is accepted that this is fairly compatible with the spacecraft.

DR. QUIMBY: One of the chief concerns among the engineers is that ethylene oxide requires eleven hours of treatment, followed by two hours of purging with a sterile gas. If we have to enter the capsule and remove the shroud for any reason whatsoever, then we have to repeat the whole treatment. And if you do this very many times, you lose your launch window.

This has been one of the principal and justifiable complaints that we have received.

DR. CHERKIN: The time factor?

DR. QUIMBY: The time factor. Every time you go in and repair a device during count-down, then you have to resterilize because there is no provision, there is no polyethylene bubble technique, there is no germ-free

technique, no black box technique for getting at repair except to contaminate in doing so.

DR. CHERKIN: Would it be feasible to put the capsule in a pressure chamber, the whole thing?

The reason I ask is that in Germany they are using much shorter cycles on ethylene oxide sterilization, by using it under pressure. In hospital techniques in Germany now they are using as much as -- I heard one claim of 45 minutes. I have a hunch this is just a straight ethylene oxide portion of the cycle. But I think you can get overall cycles of less than two hours by using pre-evacuation, 100 percent ethylene oxide and pressure.

DR. QUIMBY: How much pressure is that?

DR. CHERKIN: I think they only use four or five atmospheres.

DR. QUIMBY: Mr. Chairman, I wonder if Dr. Phillips would have a comment on this.

DR. HOBBY: Dr. Phillips?

DR. CHARLES PHILLIPS: It is about five or six atmospheres total. You are not using the pure ethylene oxide at room temperature. You can't get but about two atmospheres. This is absolute.

We have not worked ourselves at such high pressures. You have seen the equipment at Berkeley.

DR. PERKINS: Yes. It is known as the sterivit process. In fact I think there is a U.S. patent.

DR. CHAS. PHILLIPS: Yes.

DR. HOBBY: What do the concentrations run? Would it be in milligrams per liter?

MR. VARGA: We are talking in the range of 1500.

DR. CHAS. PHILLIPS: It is approaching the saturation of ethylene oxide, which is 1800.

MR. HASTRUP: At what temperature are they running in?

DR. PHILLIPS: Normal.

MR. HASTRUP: Say 70° F.

DR. PERKINS: Essentially room temperature operation.

DR. CHERKIN: They use it in the heart-lung machine, for example.

MR. HASTRUP: This would indicate they have reduced the time by a factor of about 3 for the same temperature, at the same temperature. Isn't it normally required that it is exposed about six hours?

DR. QUIMBY: About eleven.

MR. HASTRUP: We use a longer time because we have found we get skips running at 8, 9, or even 10 hours. So we have run our time up to 11 hours.

DR. CHERKIN: This will depend on concentration, humidity and other conditions. I am trying to say that I believe techniques are being used that are considerably shorter.

DR. HOBBY: I think we would run into a problem. If we use the nose cone for the gas chamber, then we cannot permit the pressure to exceed a few inches of water. We would probably have to put the whole assembly into a large chamber.

DR. CHERKIN: That was my first question.

Is it feasible? I don't know. You would have to stick the whole works into the pressure chamber.

DR. HOBBY: It would depend on how much time we would lose in actually taking the entire unit from the fixture it is mounted on and putting it in the oven and moving it out again. It might be possible.

DR. CHERKIN: Another alternative to reducing concentration, instead of using pressure, use 100 percent ethylene oxide. Just be careful.

MR. HASTRUP: This is a safety problem. I doubt if we can go in that direction. I think it is much more feasible to put the whole thing in a pressure vessel. In fact, this would probably serve another purpose.

It has been recommended that we first evacuate the air around the spacecraft in order to facilitate the introduction of the ethylene oxide. If we had a suitable chamber then we could do that as well as pressurise the ETO during exposure.

DR. PRICE: Is it not true that the information we have available currently as to the compatibility of ethylene oxide in spacecraft materials is based upon the mixtures with freon and carbon dioxide? I think we would have to initiate a whole new program looking at the effects of the pure ethylene oxide. It is highly reactive and incompatible with many materials when it is in the pure form. This would push us back in time.

DR. HOBBY: I wonder if we can get down to the next item.

DR. QUIMBY: Mr. Chairman, before you do that, I wonder if there is anybody here who can compare the efficiency of propylene oxide

with ethylene oxide for our purposes.

DR. HOBBY: Dr. Phillips?

DR. PHILLIPS: It is about one-tenth. There are two reasons for going to propylene oxide. First, propylene oxide was available and the other wasn't. The second thing is that the food people much prefer propylene to ethylene because of the fact that you get glycerine instead of glycol as a possible by-product left in the food, or something like that, and you get a cleaner bill of health from the Pure Food and Drug people. Since you are not worried about the edibility of these, and since there is plenty of ethylene oxide, there is no particular advantage in going to propylene.

DR. BRUCH: I think the figure you quoted of one-tenth is ethyl bromide.

DR. CHAS. PHILLIPS: Yes.

DR. BRUCH: Propylene oxide, the work we published on it in foods, we did the same type of procedure you did. It is a third to a quarter as effective. Of course the penetration is much lower.

DR. HOBBY: I think we will get into further discussion of the gas procedures in some of the following items.

The next area on the agenda is the tolerance levels, which has been called out in the guidelines. The tolerance at 10^{-4}. The probability is that after sterilization procedures have been applied to the spacecraft, there will be a single viable organism remaining. We have called that to be 10^{-4}. I have on the agenda a discussion of this. However I think probably the most important approach is to assume that we have to meet a high tolerance and to discuss what the chances are of

achieving these high tolerances, rather than saying too much about whether or not we should have a high tolerance.

This subject has been discussed now for two years. The original numbers derived by the WESTEX Committee were as low as 10^{-6}. We have reduced this by two orders of magnitude and still feel it is a safe number. There is a question as to how meaningful these numbers are.

The meaningfulness of the number undoubtedly depends upon whether or not it is achievable in terms of practical exercise.

I think that for the discussion today it would be more important to talk about procedures and sterilization techniques from the standpoint of achieving the highest possible tolerance. If anyone cares to comment on 10^{-4}, there might be a few short comments on it.

DR. REYNOLDS: Am I interpreting that right? I would interpret it that one out of 10,000 landings, impacts on Mars, would contain an organism. Is that right?

DR. HOBBY: It can be said that way.

DR. OPFELL: How would we go about establishing as to whether or not we have met this tolerance?

DR. HOBBY: That is under Item 7. However, if you want to raise that point now we can discuss that first. Perhaps it is more appropriate to raise that question now.

DR. OPFELL: I have raised the question. I will comment that we are going to have to come to some uniform agreement as to what constitutes a reasonable set of tests both before and after the

sterilization program.

DR. QUIMBY: To know whether you have accomplished your objectives.

DR. OPFELL: Yes.

MR. PRICE: I think people who would like to see the limits slided to one side or the other have interpreted it to mean out of 10,000 organisms per capsule, per vehicle, one of them would be alive. It is a slightly different twist to the other comment.

DR. HOBBY: I wouldn't interpret it that way.

DR. PRICE: Some people like to believe that. It would make it exceedingly difficult.

DR. HOBBY: We would expect to have more than 10,000 organisms per spacecraft.

DR. PRICE: Exactly.

DR. HOBBY: It is really what the probability is that no matter how many you have, after applying the sterilization technique you will have one remaining.

Does anyone have any comment on Dr. Opfell's comment?

DR. BRUCH: In looking at Jaffe's remarks here I feel that we were concerned with the dry heat phase of this, and we were at one time shooting for 10^{-6}. This isn't too hard to reach in dry heat sterilization. You set yourself a supposed level of contamination and carry your base time D valued calculations, go out so many D values beyond your level for contamination. If we are sterilizing the components so there is a probability of 1 to 10 in a million, yet when they go to the assembly operation and the possibility that the assembly operation is only 1 in 10

of being sterile, I think it is kind of ridiculous to have your components at 10^{-4}.

When I was reading Jaffe's report and I kept seeing 10^{-1} under these various chemical procedures I felt it was ridiculous to carry the dry heat out to 10^{-6}; 10^{-3} would be good. It seems that the weakest link in the chain is going to be those chemical methods and the sterile assembly operations. If they can't get those out farther to 10^{-4}, then I think this is in trouble.

DR. QUIMBY: There would be no sterile assembly operations if you sterilize the craft in toto by heat.

DR. BRUCH: At the last stage, yes. But if you are going in with all these sterile subassemblies and at the end you are going to have a glove box operation, that has only a probability of 1 in a 10 of being sterile. I don't think you are ever going to reach your objectives of 10^{-4}.

MR. NOWITSKY: The heat sterilization process will then have a higher degree of probability of success because it has less of a job to do, because of the internal sterilization.

DR. BRUCH: Still,if your final operation is only 10^{-1} or 10^{-2} probability of being sterile, I just can't see having the component or subassemblies out that far.

MR. HASTRUP: I think what Al has in mind is that the heat sterilization operation would be applied to the whole spacecraft after the other operations, and that the contamination level would be low before the heat operation; therefore we would have a better chance of success.

DR. BRUCH: I see.

DR. HOBBY: One could determine the decimal reduction times for different types of sterilizing agents. One might sterilize very effectively in that manner. However, there is always the difficulty of applying the actual technique. In other words, you can establish the D values or the F values for heat, for chemicals, or gas in the laboratory under test conditions.

But then you have the problem of applying this technique under actual field conditions where the conditions may not be quite the same, and where there is a higher chance of human error entering into the application.

The question is, How do you then verify your techniques under actual field conditions when you can only do a limited number of tests. If you are aiming at some tolerance like 10^{-4}, and you want to verify statistically that tolerance, you would have to do 30,000 tests, at least 30,000 successful tests, to verify this to a 95 percent confidence limit, or 46,000 tests to 99 percent confidence limit. That is certainly impractical.

One of the things that I would like to hear today is what are suggested solutions to this problem.

DR. OPFELL: One of the things we have to take in mind as the point of departure is that in the Mill Standard 105-A, for example, which is tables for testing and sampling by attributes, there has been very careful consideration given to sample size and levels of reliability and levels of confidence that one can use in interpreting production processes in statistical control. We really should start from this standpoint.

There are certain things that one can do to increase one's efficiency in terms of sample sizes by using such things as the restricted sequential sampling procedures where you decide beforehand what level of confidence you need and then design your experiments as you go along to carry you to that point.

If we do anything less than what constitutes just simply the basic statistical logic of the thing, I think we are deceiving ourselves to an extent. I keep thinking about the report of Halpern and Cornfield on the assay of the vaccine for viable virus content, in which they went through the probability of calculation of a viable organism in a particular volume of material. This is very much like this. They came to some very large samples that would be required to demonstrate that the organism was not present. The general conclusion that was indicated was that one has to think in terms of his processes before he starts to demonstrate them. In other words you cannot inject quality into a process in the final analysis. You have to build it in.

This is why we are concerned with this business of having components that have a high degree of reliability with respect to sterilization so that at any point we can reduce the amount of inspection, remembering that these things are all tied together. I am putting input from all directions.

The point is that any particular final sterilization process, the effect of this sterilization process will be a function of the entire history of the components that come in.

I think, for example, of ethylene oxide as a sterilization process. One could speak of it as being effective in two hours, six hours,

or just simply not being effective. It depends almost entirely on the previous handling procedures. One can conceive of handling procedures which will make it impossible to sterilize with ethylene oxide. I think in terms of where you put some protecting film over spores, they just inhibit it. I don't think we can talk in terms of F values for an ethylene oxide process for sterilization unless we can define specifically what has gone on beforehand.

In summary we have two aspects. One, we have to recognize the statistics of the situation for a well and thoroughly defined set of physical parameters; and the other is that we are going to have to define the physical parameters that are compatible with our end result.

DR. HOBBY: Dr. Perkins, do you have anything to contribute?

DR. PERKINS: I am a relative newcomer in this field. There is so much food for thought here it is very difficult for me to express an opinion. I am picking up a lot of information here, little by little, I guess.

I am wondering, in the technique that is used here, and the organisms that are selected, what happens to the base in terms of heat resistance, whatever method you are using for sterilization. Supposedly it is dry heat we are primarily concerned with. It seems to me that one encounters great variations in organisms and how they are grown and that sort of thing, and their characteristics and the populations used and so forth. I would be very concerned about any data unless I had a base upon which to establish the resistance of those organisms in the beginning. Perhaps this has been done.

DR. BRUCH: I think the program that we had at Wilmot Castle was doing that. We were screening microorganisms for the dry heat resistance. I don't say we covered the panorama of organisms. I think we established that an organism which has moist heat resistance does not necessarily have dry heat resistance.

Then we started, based upon this business of soil samples, which people will always keep tossing at you, we have gone to artificial carriers and found that the resistance on a piece of paper is not the same as a sand sample. Why this is we can't really figure out. In the vacuum work we found we reduce the resistance on sand by putting the material under vacuum, dry heat.

Maybe Dr. Wynne has some comments. He has worked on heat resistance of organisms for sometime.

Does your group have any data on this area that lends support?

DR. WYNNE: A logical extension of what you have just said, the substrate on which one does the test is very important. A logical extension of that is that the resistance of organisms in a sealed component, such as a capicator, would be not necessarily the same or even closely related to the resistance on a piece of filter paper, vermiculite or what-have-you. So I think that the only completely satisfactory way to arrive at these estimates is to deliberately contaminate the components during manufacture and then test the procedures, which is the approach we are using.

Dr. Bruch, you have the best data for that, however, to support our approach of anyone that I know of.

DR. BRUCH: What we have done is started organisms in solids and in components and we find that the resistance has gone up remarkably. In fact, in solids we have picked up extremely high D values. Part of this we can attribute to heat penetration. We are working with small samples going to test groups. We were amazed at the way the values climbed that high. We were doing work with solids. What got us into this was the work we were doing on solid propellants for Thiokol Surveyor. We found that the figures were within what we were getting for putting spores in sand. In some of the other solids we have gone to dental cements, and dental plastics, and this sort.

I would say Dr. Wynne's comments support it, that you have to have a resistant organism that you know is resistant by laboratory tests and then put it into the item that you want to sterilize.

DR. WYNNE: That is precisely what we did.

We did this on a theoretical basis partly to be sure. Dr. Bruch's group has provided the necessary experimental evidence better than any other group I know of.

DR. QUIMBY: Do you mean that if the organism is entrapped in a spacecraft component of some type or other, that it has a much higher resistance than it has in soil?

DR. BRUCH: Well, no, not quite that high. Of course soil-- This is working with known organisms. We have never taken known organisms and put them back in sterilized soil. You just don't get enough data that is meaningful. You get high resistances but you can't interpret what is causing it.

I would say the levels we got of materials entrapped in solids is approaching the levels we have needed for sterilization of small soil samples. But I have always felt that using soil samples as a guideline in this work, we are setting standards too high, except George claims he is goint to have roughly two grams of soil on the final spacecraft. I don't really --

DR. HOBBY: You shouldn't have quoted me on that.

DR. BRUCH: Let's say dust or something of that order. We have tested dust and it isn't as high as soil. I think definitely the way the organism is entrapped in the soil sample has a lot to do with the mineral, clay mineral and things of this sort. I thought I was getting too far away from what was involved here in component sterilization, so I went over to the solids angle of plastics. We were led into this through the works on the solid fuel for Thiokol.

MR. HASTRUP: Although we try to keep the spacecraft clean, I think we have to accept the fact that it isn't perfectly clean and there might be something like two grams of soil or dirt or dust on the spacecraft. In that light it would seem somewhat realistic to use soil or dirt samples as a realistic test; at least if we can't find something else that causes the bacteria to be more resistant.

We are also looking at dust samples in our ethylene oxide sterilization testing now because between the time we might heat something and gas sterilize it, there can be quite a bit of dust settling on the spacecraft. There again we have found it more difficult to sterilize the dust than we would isolated organisms on particular substrate materials.

DR. PERKINS: You assume all organisms reside in strictly an anhydrous environment?

DR. BRUCH: We didn't try to play around with the levels of moisture involved. In the food field there is this comment about superheated steam being a dry heat sterilizing agent. Let's say we think that in some of this entrapped work we were doing in solids, if your moisture content was high you got a little faster killing.

We didn't control it to any extent.

DR. QUIMBY: Mr. Chairman, I would like to ask a question of Rolf Hastrup.

A few weeks ago we learned from Carl Sagan that the Russians had used formaldehyde and high pressure steam in sterilizing Lunik II. Are these two approaches incompatible with the experimental devices on the spacecraft?

MR. HASTRUP: We don't have much information in that respect since we have never used these on spacecraft.

DR. QUIMBY: Is the formaldehyde corrosive?

MR. HASTRUP: We have been using formaldehyde in liquid form. If it is left there long enough with moisture it appears to be quite corrosive. Possibly Dr. Opfell could comment further.

I would like to make another comment, though, to that question.

It would seem that these techniques would apply only to surface sterilization. They would not accomplish internal sterilization of the spacecraft.

DR. QUIMBY: How about the superheated steam?

MR. HASTRUP: As far as the actual temperature is concerned, this may tend to sterilize. But superheated steam is usually used to sterilize at a lower temperature than dry heat sterilization.

DR. QUIMBY: This is essentially autoclaving, isn't it?

MR. HASTRUP: Yes. But autoclaving only guarantees sterilization at the surface because the steam, which is one of the active factor, --

DR. QUIMBY: Does not penetrate crevices?

MR. HASTRUP: It will penetrate into some crevices but not into the interior of hermetically sealed components.

DR. REYNOLDS: It depends on the duration, doesn't it, of whether the components are heated up?

MR. HASTRUP: The steam would never get through. The temperature, whatever that might be, would soak in, and if it is high enough to accomplish dry heat sterilization, it would be effective.

DR. PERKINS: The total effect would be that of dry heat.

DR. REYNOLDS: Yes, if long enough.

DR. BRUCH: They were adding the methanol at the same time as adding steam?

DR. QUIMBY: Methanol?

DR. BRUCH: I mean formaldehyde.

DR. QUIMBY: I don't know, they were probably combined procedures. We don't have the details.

DR. PRICE: Formaldehyde vapors have very poor penetrating powers.

DR. OPFELL: This is true only at temperatures below 80°C. Above 80°C. formaldehyde is highly penetrating. It would be better than ethylene oxide.

DR. BRUCH: In terms of penetration?

DR. OPFELL: In terms of penetration, in terms of effectiveness. But at the same time it is highly corrosive in the presence of steams. Your compatibility problems have to be carefully scanned.

DR. PERKINS: I think you would need a lot of data to prove that point, that it is more effective than ethylene oxide below 80°. I think you would need a tremendous amount of data.

DR. OPFELL: This is data that I have personally collected and reviewed over the years. It is proprietary information.

DR. CHARLES PHILLIPS: Our experience on penetration hasn't been anything like that. The reason we thought particularly of ethylene oxide, it does go into grease films and oil films which offer protection against aqueous formaldehyde.

DR. HOBBY: Will you speak up, please?

DR. CHARLES PHILLIPS: Ethylene oxide was more penetrating than formaldehyde. We didn't have the sterilization stopped by a film of grease or something like this. These were used -- Briggs has more on this -- in cabinets and things like this, sterilization cabinets. We have had quite a bit of experience with steam and formaldehyde. I would still question the penetration.

DR. OPFELL: The thing that I have observed with formaldehyde, for example, is that it penetrates material like polyvinylchloride of substantial thickness which ethylene oxide normally won't go through

in normal sterilization times. Formaldehyde will not penetrate equivalent thickness of polyethylene, which ethylene oxide will go through. You have to carefully define the particular situations.

There are instances in which formaldehyde is superior, and instances where it is not.

DR. HOBBY: I wonder if we could get back to this question that Dr. Opfell raised a mement ago on how do we verify the sterilization tolerances, or sterilization procedures.

Is there anyone else who would care to comment on this?

This is something we do have to face. The numbers are meaningless unless we can express these numbers in terms of operational procedures.

What constitutes the verification of an operational procedure in terms of a tolerance number? How can we say, how do we verify the fact that after we have applied a given sterilization procedure that we have indeed one chance in 10,000 that a viable organism still remains?

Are there any good ideas on this?

DR. BRUCH: I think Dr. Opfell can comment on the pharmaceutical industry, in terms of filling operations. As I understand it, they just take so many samples out of every batch of a thousand filled ampoules and test it, and derive their figures that way.

DR. OPFELL: No, you do this on the basis of the normal hypothesis testing. You set up a normal hypothesis that the process is capable of producing a continuing stream in which lots are all of the same quality. You do not proceed to sort out good lots from bad

lots. Each product will have specified for it certain procedures which are acceptable for sampling tests. These are filled and those from which they have recovered these specimens should not be in any sense different from those lots in which they did not recover organisms.

What you do is decide before you draw your sample what level you are going to accept in terms of contaminants on which you are going to decide whether or not your hypothesis, the products, is in statistical control, that it is acceptable. The simplest way to think of it is where you are autoclaving something like several thousand bottles of intravenous injections. According to law the entire lot must be sterile. You are not by any stretch of the imagination going to be able to follow something like U.S.P. tests and draw ten samples of these lots and on the basis of these ten being sterile say that the rest of them are. You do know that if there are any of these that are not sterile that this is inconsistent evidence with the rest.

From the standpoint of the test of statistical hypothesis, combine this ten on this lot with the ten on all the other thousands of lots that have gone before that have been done in exactly the same procedure, then you have evidence on which to say that the product continues to be of the nominal quality.

The thing that is most to bear on the thing is not the results in this particular set of ten but on the continuous sequence of successful operations. Once you break this sequence then you have a real problem to face. You have to develop an engineering understanding

of how this sort of thing could have happened. There must be some systematic error that has crept into the process. But it isn't this ten samples on a particular autoclave load that decides whether or not the lot is sterile. This is my contention.

The reason I raised the question initially is that we are not going to be able to take the first spacecraft. We can take a small sample of this. We are going to have to have established our procedures, we are going to have to know in quantitative terms the reliability. This I don't really see much way around. It is just a lot of work and a lot of careful thought in terms of the design of the tests.

DR. HOBBY: How do you establish these procedures? We have a limited number of spacecraft. We can only do a limited number of sampling. It seems that the only alternative is to work out the procedures on a laboratory scale. But then there is the problem of extrapolating that under actual operational conditions.

DR. OPFELL: Yes. One has to identify this into elements to the extent that you can. There are certain portions of this handling procedure which you can standardize and which you can develop experience in the laboratory. For example, the sterilization of, say, a capacitor. You look to hundreds of capacitors, whatever it takes to develop the level of confidence that you need for capacitors, and for the other components that you are going to use, as being sterile inside. Then you are left with the question of sterile assembly.

The sterile assembly operations are going to have a lot of similarity. Whether or not it is the screwing of a capacitor

together with something else, or making a solder joint, these you can identify in terms of elements.

DR. HOBBY: Suppose you have 10,000 different types of parts and you have to do a sterile assembly with these 10,000 different types of parts. Do you think then it would be practical to work out standard procedures in doing a dry-box procedure with different types of parts?

DR. OPFELL: I think you must work out a standard procedure. You must identify to the minimum of common procedures, that is, elements that involve similar physical arrangements, similar materials, with similar handling.

The extraneous parts of this are the parts that are extraneous to the sterile assembly operation, the mating and closing of bacteria, is not and cannot be the same. Those elements which directly affect the sterility must be the same. One can identify the minimum of these.

DR. HOBBY: I would like to ask you, Are you implying, then, that we would have to do 30,000 tests per part, or at least wherever there is a difference in the procedure, to statistically verify a given method?

DR. OPFELL: I don't think you are going to statistically verify a method. What you are going to do is to develop a procedure and you are going to have a hypothesis that this procedure is capable of doing the job.

Then what you are going to want to do is to verify whether your hypothesis is true or not. The amount of testing that is required to verify this hypothesis depends upon a lot of other factors. One of them is the certainty of the physical and chemical biological principles that

underlie the procedure. The second thing is the number of factors that can come into play to affect the procedure.

After you have developed the procedure, and you have determined the level of confidence you want, then you pretty much depict the number of samples that you are going to have to do. There isn't much flexibility there. So the place one would have to work is on the input. This is why one usually thinks in terms of reducing the initial innoculum. If you want to get a sterile result, the cleaner the material you work with the less testing you are going to have to do to confirm it. You are going to find by the first -- You are going to be able to develop a sequence of successful tests more rapidly than you will if you start with a heavily contaminated one.

DR. HOBBY: It sounds as if you are saying that we have to proceed partly on theory and partly on actual tests. Is that correct?

DR. OPFELL: Yes.

DR. HOBBY: Dr. Phillips, have you anything to say on this matter?

DR. CHARLES PHILLIPS: I think that Dr. Jaffe's document is an effort on his part, I think, to look into these various procedures and put numbers to them. I think he has done a masterful job. I am quite sure that every single person around here can pick one or two numbers and probabilities and argue about them until the cows come home. I don't see much point in it, because they are -- most of them, a lot of them -- a matter of expert opinion and not subject to test. He has, as Dr. Opfell is saying, looked at the various things which are common and tried to put numbers on them. Knowing about sterility and the absolute term,

there is always a question of whether or not you have arrived at that.

I think it is an excellent document and he saw a lot of people I know all over the country in preparing this, and there is an awful lot of good thinking that went into it.

You have in essence here a document which does outline what he considers the various steps that you have to go through, and outlines what he thinks the chances are of coming up with something. This 10^{-4} is a pretty good figure which he thinks is feasible.

No, you don't have to take 30,000 spacecraft and test each one. But tests have been done on various things. There are hundreds and hundreds of components. They have elements of similarity. And you probably only have to pick one or two. There are things sealed in ceramics, things sealed on metal surfaces, things embedded in plastics. This is the type of thing, the heat thing, internal thing, that Bruch is doing.

DR. HOBBY: Dr. Cherkin, do you have any comments on this?

DR. CHERKIN: Yes. Dr. Opfell mentioned practices that have been used in sterilizing certain solutions.

I don't think they all fall into one category. I would like to say that I agree with what Dr. Opfell has said. Sterilization is a matter of, we would say, production. You achieve sterilization by what you do. You get some measure of verification from the tests. You have to do the tests. If I were given ten bottles or a hundred bottles out of a lot of five thousand and had them run through and found that these were all sterile, just on the basis of that information I couldn't say that any other one was sterile. I would have to know the whole history.

This starts out with, incidentally, with the initial design of the product. Let's just talk about the sterile product.

When you are working out a sterilization procedure, as we all know, you have to design the product to be sterilizable. That has to be built into the design requirement right from the start, just like any other requirements. You can't just get it made and decide let's now think about sterilizing.

And so the materials, and the production methods have to be designed with sterilization in mind.

I am trying to do two things: think of sterilization from the pharmaceutical field, with which I nave been familiar for a long time, and sterilization of the space vehicles with which I am completely unfamiliar. This is a little hard to do. Maybe I ought first to go through pharmaceuticals quickly.

When you have a new product that has to be sterile, that is one of the considerations that you have in mind right at the outset, both of the product and its container. You design a sterilization process that you think will work for it. And then you verify it, and we use soil, what we call a standard soil, if you will accept that term -- you don't have to. We call it that. It looks like something you dig up out of a garden, but that is what it is.

We run a great number of tests while we are verifying the procedure, just as you have indicated, until we are satisfied that our process will sterilize the product even if it is contaminated beyond all conceivability of contamination in actual use. Then we go on the assumption that if this process will work on a tremendously contaminated sample -- and

this is religiously carried out and controlled -- then it will work regularly.

Then we use all kinds of controls on sterilizers and we run temperature distributions on them and we run routine culture sterility tests. We are very sensitive to all the details of those tests, including temperature and air flow in the incubators and all the other things that you have to watch.

We are using terminal sterilization along with all these other things. When I say "we," I think everybody in the industry does it.

If you are going to try to-- Let's put it this way: I think we are all agreed that an effective terminal sterilization would resolve a lot of kinds of problems that you are talking about. I realize that there is a great big fat "if" on that. But while we are on that "if" one could conceive of the possibility of putting soil samples in secluded portions of the vehicle so that they could be snagged out aseptically, and then if you ran a sterility test and found that those were sterile, you have a pretty good measure of confidence that the rest is sterile.

I think what you have done is broken the problem down into as many individual components, each of which is so-called terminally sterilized. You have a component and heat sterilize it and you keep it wrapped up, and you keep it as free from contamination as you can. I thoroughly agree with this business of the fewer bugs you have the easier it is to kill them, indeed.

The more individual assemblies that you have to do sterily, the greater your problem is, and something tells me it goes exponentially.

In a nutshell, I think you have to get as close as you can to

controlling it with soil samples.

DR. QUIMBY: What kind of terminal sterilization do you use in the drug industry?

DR. CHERKIN: We prefer to use autoclaving.

DR. QUIMBY: What do you do with heat sensitive products?

DR. CHERKIN: Then we use gas, ethylene oxide. This is generally used. Formaldehyde has some practical disadvantages, although it has been used.

Radiation is used, particularly in England, for example, disposable syringes. They are sterilized by radiation. From what I have seen of the effects of radiation on plastics, I would be a little bit concerned about what it may do to your components.

DR. QUIMBY: How about the effects of radiation on your drug products?

DR. CHERKIN: I think by and large this is not a problem.

DR. QUIMBY: It is a problem in spacecraft sterilization. Radiation as a sole means of sterilization is a problem in spacecraft because some electronic components are radiation sensitive.

DR. REYNOLDS: Are you speaking of ionized radiation?

DR. QUIMBY: Yes.

DR. BRUCH: What about something like a vaccine, where you can't give a final heat treatment or something like that, which is put up in individual ampules. What confidence could you have in procedures like that?

DR. CHERKIN: Very little. We are in the biologics field.

DR. OPFELL: This is something which is given a lot of very

careful consideration. Lederle Laboratories I guess puts in about as much work on this particular area as any one I know. Their people are right in the forefront of the science of statistics, too. They just simply must be, because there is a lot of dollar bills that are involved in vaccine production simply in developing efficient tests to decide this sort of thing. Most all vaccines that are tested for what they call safety, that is, the presence of organisms produced in the vaccine, involves testing in animals. If you are testing something like a cow vaccine, or something for humans, you just can't afford any losses. Consequently, you have to draw up your test to be highly efficient.

DR. BRUCH: Do they worry, on seeing vaccine producing samples, about extraneous organisms getting in?

DR. OPFELL: Yes they do.

DR. BRUCH: If you don't give a product a final terminal heat sterilization, just what kind of confidence do they have that the product is sterile, which is what I think is going to be the space probe thing. You aren't going to be able to give it a final terminal sterilization. At least it appears to me that you can get a lot of the subassemblies but still you are going to have --

DR. HOBBY: We are aiming at the final terminal sterilization.

DR. CHARLES PHILLIPS: Vaccine production is a bad example to use because there you are fighting two problems. One of them is, you want to maintain as much antigen as you possibly can, and yet still be sterile. So they are coming up, all the sterilization processes, either heat, formaldehyde, ultraviolet, are destroying the antigenicity. So

you want to stop rather close there. This is why this fantastic statistical thing -- you don't double to get a margin of safety in vaccine production. You add two percent.

DR. BRUCH: I want to get around to the polio virus. I was more interested, at the time they were filling the polio virus into the ampule, that they weren't getting organisms or matter dropping in out of the air.

DR. OPFELL: In general they will use a preservative.

DR. BRUCH: In other words, they are developing a level of contamination and controlling with a preservative which is not possible to us.

DR. OPFELL: The body has a defense mechanism. You can take certain doses.

DR. CHERKIN: This is one of these things that gives me a headache when I try to think of the pharmaceutical problems and these problems in space, and this is right. And there is another factor. I don't know that anyone has ever received a sterile injection because an injection implies going through the skin. I am very doubtful that anyone has really sterilized skin. This is a factor, and this is quite right. If these B subtillis niger, God forbid, fall in there, and you have a preservative and you keep it at a temperature that inhibits growth, it won't grow out.

You are always going to be putting one organism into the circulating blood. In fact, bacteria is a normal condition.

But this is completely different, and that is the trouble here. This is completely different from this problem where you don't want one spore in the whole thing. I would like to make one other comment.

On this point of vaccine production, I thought, too, Dr. Phillips, that this was completely different. I think there is a general problem.

You can sterilize space vehicles if you put them in a furnace. Of course when you take them out they are no longer a space vehicle. You can say this. But they can be sterilized. You may end up with a reliable factor of 10^{-10} or 10^{10}, however you are using it. I think this is a generalization, that in sterilization two things are going on. You are destroying organisms, viable organisms, and you are destroying the product.

There is a fellow at the University of Wisconsin in this not very much recognized paper but which I thought excellent, in which he plots the competing curves and explains the kinds of conditions that you need to choose, in which you have this competition between the destruction of the organisms and destruction of the product. Isn't this what you are doing?

DR. QUIMBY: Exactly. This is the whole problem, particularly with either heat sterilization or ionizing radiation, and even with some fluids.

DR. CHERKIN: I also regard, although I was considerably depressed by this, although I thought it was excellent, which points out more of the problems, this is one comment that I had. I would be hopeful of finding something analogous to this business of the trend of destruction of the product versus the trend of destruction of the organism. For example, this business of using 125° for 24 hours. I realize that at 190 in some of your components they may just melt, blow up or do something dramatic. I think this is something that has to be, or ought to be explored.

Very often a short high-temperature procedure is preferable, oddly enough.

MR. HASTRUP: Of course, to get into short high-temperature cycles we have another special problem there because we have large spacecraft. Many items on there which serve as terminal insulators. While we would like to heat at two hours at a higher temperature, to get the whole thing up there would possible take another six hours and we would defeat our purpose.

DR. CHARLES PHILLIPS: The heat sterilization is an area of low dry heat that had been considered highly impractical and was never explored and so there is very little data. We were asked by George's group about two years ago about this and we pulled out not much, because very little work had been done. With heat, with microorganisms it is a time-temperature relationship. With heat and materials,very often there isn't, you see. There is a top limit. At certain temperatures things begin to melt. At certain temperatures things begin to decompose. At lower temperatures they just last more or less indefinitely. And the whole idea of getting more information on this was that if we could get reliable information at 125, there are probably a lot of materials that would take 125 almost indefinitely, you see, whereas the bacteria would of course slowly be killed off.

We hope you will add things where you have a large margin of safety with material, which isn't necessarily true of the biologicals, of course, which are notoriously sensitive to almost all kinds of sterilization. We are in mechanical things where we hope we can pick processes which have a nice margin of safety on the effect of material. This means all the materials have to be tested for compatibility to sterilization techniques.

MR. VARGA: I think we have to add impetus to what Dr. Bruch

said and Dr. Opfell, and also Dr. Cherkin and Dr. Phillips. You have to take each individual process that we are going through in the particular program that we are talking about, and determine what our probability is for that particular assembly procedure in the spacecraft design. This is what we have done on Surveyor. We run into various problems where we start off hoping that we can sterilize everything by the heat process. We find that we do have heat-sensitive components so we have to resort to other type methods. This is where we get into the process of possibly accomplishing a certain procedure of sterility through 10^{-3} for one process, and trying this into something else such as your heat process where we have a much greater probability. Then when we sum or multiply this together, our overall probability will come up to be the lower value. How we arrive at 10^{4} is beyond my analysis at this time.

DR. HOBBY: My feeling from what has been said so far is that the 10^{-4} tolerance really doesn't have too much meaning; that we have to proceed on the basis of using the best techniques available and designing the techniques as carefully as we can. Actually getting a number for a particular procedure seems to me, from what has been said, not practical.

DR. OPFELL: I would like to make one additional inquiry to sort of underscore Dr. Cherkin's comments about starting sterilization at the initiation of design. It seems to me appropriate that for your type-acceptance tests for all components that you really should perform these on those components which have been exposed to your sterilization processes. The sterilization is an essential manufacturing process in the preparation of this component just the same as any other element of the manufacturing process, and it is only those components which are sterile whose reliability

is worthy of consideration in terms of your spacecraft.

DR. CHARLES PHILLIPS: I couldn't agree with you more in what we are going to do in the pure necessity to use the best methods we possibly can and don't ruin the spacecraft, and that is it. There existed of course this 10^{-6} figure, and I have gotten into more arguments as to how this was selected that I don't want to get into.

What Jaffe has really done, as I interpret this, he has looked at our best methods, and with as good a method, to put a figure as he can, he said what we can do right now is 10^{-4} and not 10^{-6}. This, I think, seems to have aroused no horrible thought. 10^{-6} was a figure, sort of wishful or hopeful thinking. He said what we can do is something like 10^{-4}. I think this is a very worthwhile achievement. As far as I can see, no one has gone into fantastic shock at the 10^{-6} as not possible. This is my feeling as to it.

DR. HOBBY: 10^{-4} is something to aim at. It would be desirable to have some standard which you aim at. In other words, what is an adequate sterilization procedure? You set up some kind of a standard and call that adequate. It is just that we don't want too little sterilization. We would like to have as much as we can. Perhaps setting a number would help that.

MR. HASTRUP: I agree with you in the comment that you must try merely to do as well as possible to some extent. I think that the number is directly useful in some applications. For instance, if we are going primarily to heat sterilization, then in establishing D values, we can use those to estimate the required temperature cycle that will give us the confidence you want to achieve 10^{-4}. In some cases such as providing sealed

enclosures to maintain this, it is going to be impossible to actually test and determine that this is what you have. You are going to have to merely do as well as possible to make that seal perfect. But in establishing the basic processes of ethylene oxide and heat, I think we can utilize the 10^{-4} number with our D values in coming up with the required cycles.

DR. HOBBY: That is true, but you can't completely verify the technique.

MR. HASTRUP: There is still some element of doubt. There is a confidence limit that we must achieve.

DR. REYNOLDS: Does this automatically mean that an experiment that was not for some reason capable of sterilization to the level of 10^{-4} would not be included in the spacecraft.

MR. NOWITSKY: It should, but probably doesn't at this time.

DR. HOBBY: That is a decision for headquarters to make.

MR. HASTRUP: Again I think we should be consistent. If we are going to permit, knowingly, procedures that would be less than good for 10^{-4}, then why should we break our necks to heat things higher to get to 10^{-4}? I think that we should be consistent and choose some number that seems practical enough and actually use that as the goal.

DR. REYNOLDS: That is another reason for having such a number so that you can use it as a cutoff.

DR. WYNNE: I was going to raise a fundamental point here. Dr. Cherkin certainly made an admirable distinction here, very well drawn, between contamination of pharmaceuticals and vaccines and the problem posed in Dr. Jaffe's report. Certainly certain numbers of skin spores have been shown to be contaminated but no one really worries whether or not there

are one or two B subtillis niger spores in the vaccine, because the body can certainly overcome these with no trouble at all, and there is no problem.

We think one organism landing on materials will create a problem. I don't think this is necessarily a hard and fast distinction. In the first place, one organism might fall in certain parts of the earth and not multiply, but what matters, we have a different problem altogether. Unless we just accept Sinton's work at face value that there are carbon-hydrogen linkage, just accept this at face value -- and there is some question about this -- then we are left with this sort of situation. Assuming that there is not organic material there, elementary nitrogen is present, of course, and carbon dioxide, then an organism from earth, and very little moisture, less than one percent, an organism from earth would have to be capable of multiplying at a very low humidity level, fixing free nitrogen, and photosynthesizing simultaneously.

There is an organism which can, at least there is one well known organism that can fix free nitrogen and do photosynthesis at the same time. We tried to get this organism to survive under simulated Martian conditions and failed completely. Four days is about it. It just decreased logarithmically in numbers.

As you know, at the School of Aerospace Medicine, earlier work has shown certain spore formers will survive under simulated Martian conditions, and presumably would multiply to about a ten fold level. There is grave doubt now whether this multiplication, that is, increase in numbers, I should say -- whether this is real multiplication or not. It may be a modification of the dormant state of the spores. We haven't proved this. It certainly is a very grave suspicion. It is difficult to attack this

problem experimentally. I suspect that the earlier results were either due to a change to the dormant condition of the spores or they were just living off their own fat, so to speak, in the earth's soil, which is the substrate we were using. If neither of these is true, it does not necessarily follow that such an organism would multiply on Mars, particularly one or two, or if a few, a very small number, are present in the spacecraft. Furthermore, these would have to be thrown out on the Martian landscape even if they could multiply in order to multiply.

So I don't think there is a hard and fast distinction. I think one or two organisms probably wouldn't be anything to worry about in a Martian spacecraft.

DR. HOROWITZ: Mars can fight back, too. It has some sort of resistance mechanism. Even if we assume that Sinton is correct and that there are carbon compounds on the Martian surface, and even if we assume that the entire Martian surface is not as dry as the average, -- there may be springs, there may be permafrost and actually wet regions -- there may be some very favorable spots for multiplication of terrestrial microorganisms. Still there are other factors involved.

First of all, we are quite sure that any organism that lands on Mars is going through a diurnal cycle of freezing and thawing. One thing I would like to hear from some of the experts here is just how effective alternate freezing and thawing is in killing microorganisms. It certainly kills off some microorganisms. You have to remember that we are not really afraid of landing an organism on Mars, or of even it surviving on Mars. The thing that we are worrying about as biologists is that it will multiply and change the Martian ecology. This means that any terrestrial contaminant

has to multiply faster than it is being killed by Martian conditions.

We don't know a lot about Martian conditions. We know a few things. One is that it is very close to being anaerobic. The other thing is that temperatures at night go to very low levels, and there may be regions of the planet where the temperature never gets above freezing even in the summer. On the other hand, there are regions at the Equator where the temperature gets to about 25° Centigrade, at least in the summer.

I think one thing we could use very effectively in this discussion is more information about Martian meteorology than we have now and Martian conditions. Perhaps we will have this before you really have to worry about landing a capsule on Mars.

I would like to ask what any one here knows about the effect of freezing and thawing on microorganisms. Suppose a terrestrial organism lands on a wet spot on Mars where it can in effect germinate and multiply when the temperature rises above zero, or say rises above minus 5°C. Then at night it falls to minus 100°C. And this goes on day after day after day.

DR. OPFELL: There are two instances in food and drug work. One is in frozen foods which have to be kept frozen because if they do thaw, they have a proliferation of organisms. This is frozen foods. This also applies to such things as stuffed turkeys that are stuffed and then frozen, where you have a substantial time lag from the time that the outside freezes to the time the inside freezes.

DR. WYNNE: That is a critical point, not just one thawing.

DR. CHARLES PHILLIPS: We have quite a bit of data on this. It varies as you might suspect with the organism. Once frozen -- it can keep on dropping or varying up and down -- the better off the organism is. It

is the freezing process itself that makes it a little different as to how fast and how rapid it is, or something like that.

By and large, I don't think you can say that just alternate freezing and thawing would knock out earth organisms. The tougher type certainly would not. We worry a lot about certain of the quite sensitive pathogens where this does get to be a little bit of a problem, particularly when the organism is in an environment where, even though it loses something in the freezing, when thawed again it can proliferate.

With most of the tough organisms, probably on Mars, I don't think that can take care of the question.

DR. CHERKIN: Aren't there some experimental data? I have seen one fairly recent paper on this. It only covered a few organisms, by Harris.

DR. CHAS. PHILLIPS: We have a lot of data. There are loads of data we have not published and do not particularly want to publish.

DR. BRUCH: A lot of that was done in the dried state. We worked with the dried pathogens.

DR. HOROWITZ: They won't multiply when they are dry.

DR. CHAS. PHILLIPS: It seems to be a physical phenomenon that the formation of ice crystals and so forth, disrupts the walls. When they dry, just zero degree C does not seem to be any kind of point in effecting the bacteria. Only in fairly moist conditions do you get freezing effects and so on.

I think it is almost entirely cell wall rupture, something mechanical.

DR. HOBBY: The cells are more susceptible when they are in an active metabolic state. Is that true?

DR. CHAS. PHILLIPS: Yes, sir.

DR. HOROWITZ: That is what we are worried about. No one worries about dropping organisms on Mars in an anhydrous condition. They cannot multiply, and it does not bother us. It is only when they fall in a place where the conditions are good enough so that they can multiply.

DR. CHAS. PHILLIPS: But I don't think you can count on that. Take it the other way around. I could not agree with you more than that the probability of infecting Mars and having great growth takes place with relatively few organisms.

I have used an analogy, I have used it for the first time at jet propulsion, the probability of throwing a match out of a window and starting a forest fire is very low. The thing you are worried about is if it could happen, even though the probability is low, it would be catastrophic. I agree with everybody, the probability of coming there three years later and finding Mars completely overrun with earth organisms and every one of the native life of Mars completely destroyed I don't think is high. However, I do think that as long as there remains the possibility, it is something to be quite concerned with.

DR. HOBBY: Up to now we have made the assumption that if we landed a single viable organism on Mars, we would get growth. We simply made that assumption just on the basis that we have not considered all the possibilities or that we have just wanted to be safe.

DR. QUIMBY: We have to make this assumption because we simply do not know whether or not the organism will land in a favorable environment for growth.

DR. WYNNE: I agree, but I disagree with Dr. Jaffe's conclusion that this probability is practically unity of a single organism multiplying.

I think it is much, much lower than that. It might be more of the order of ten to the minus 4 figure we are talking about.

DR. QUIMBY: You mean that a single organism would multiply.

DR. WYNNE: Yes.

DR. CHAS. PHILLIPS: I would like to make this one thought. The probability really in my mind on infection, if you want to call it that, of Mars, is whether or not the organisms were put in a favorable spot. There it does not matter. If we say there is no need to sterilize, because if the spacecraft goes down and opens up in a spring, as Dr. Horowitz talked about, one organism will do just about as bad a job as a hundred thousand or ten million. The probability there is whether it gets in the right place. If it gets in the right place, numbers just are not very important. Am I clear?

DR. HOROWITZ: You are clear, but I don't agree.

DR. CHAS. PHILLIPS: This thing usually taking more than one organism up -- incidentally we have published on the number required in the human body of Tularemia as ten.

DR. QUIMBY: It happens to be low in tularemia.

DR. CHAS. PHILLIPS: Which shows all the human resistances, so that while I think the probability of carrying on in Mars may be low, I think the main thing to determine is where it lands if you put any number in in the right place.

DR. HOROWITZ: If you put one on, it has to land in the right place. If you put 10^8 on, and the space capsule breaks open, some of them may fall in very unfavorable ground.

DR. CHAS. PHILLIPS: No doubt. But still nearby there would have to be some kind of favorable location.

DR. OPFELL: I would like to offer some observations. Two of the most unfavorable environments I can think of for microorganisms, one is in JP-4 fuel which the Air Force is having a substantial amount of problem with. They have isolated some fifty different varieties of microorganisms, most of them fungi, that are causing problems. Another area that I looked at recently was some of the granite rocks on the east side of Rock Springs. You can quickly count up to 25 different species of lichens that are growing on the rocks. If density is an indication, they go through the diurnal cycle from above to below freezing, in an extremely dry environment, and all the moisture they get is out of the air, and that is precious little. You don't really think in terms of one particular odd-ball species. There are lots of varieties that can proliferate in unusual or "hostile" environments.

DR. HOROWITZ: How much do they grow?

DR. OPFELL: I don't know what the rate is. I expect it is relatively low. The population on those granite rocks is rather large, the total population.

DR. WYNNE: I once observed a fungus growing in chemically pure hydrochloric acid. How it was doing it, I don't know. The rate of growth of course was tremendously slow.

DR. CHERKIN: We have it in ten per cent copper sulphate solution down around pH-3. We had to sterilize the copper sulphate.

DR. WYNNE: Of course if traces of fixed nitrogen are present on Mars, then one might expect phenomena like this to occur. We don't have any evidence really that there is fixed nitrogen. But if there is, such things could occur.

DR. REYNOLDS: Also, in addition to the problem of changing the Martian ecology, just the problem of recovering earth organisms in such future earth exploration is to be avoided, even if not in large numbers.

DR. QUIMBY: Even if they did not multiply?

DR. REYNOLDS: Even if they did not multiply to such an extent as to change the ecology, as Dr. Horowitz brought up, you would not want to recover them.

DR. HOROWITZ: The chance of recovering them would be very small. They would not be homogeneously distributed, it seems to me. That is a risk that you almost have to take.

DR. HOBBY: If you keep the amount of contamination down per vehicle, the chance of recovering any of them should be very small.

DR. QUIMBY: Mr. Chairman, Dr. Cherkin mentioned that you could sterilize if you used a furnace. I think that this might be the place to introduce some new data into the meeting. I will put it on the blackboard. Maybe some of the participants of the meeting would like to copy this down. This is a report from National Research Corporation, where they took two of the more resistant organisms, B subtilis niger, and Aspergillis niger, and put them in vacuum plus heat for five days. The vacuum was 10^{-10} torr, and the heat is indicated on the top line, -190°, -110°, 25°, 53°, 60°, 86°, 100°, and 107°. Survival falls off very rapidly as one gets into the higher temperatures up to a hundred where there was no survival either at 100 or at 107 degrees.

DR. HOROWITZ: What are these numbers?

DR. QUIMBY: Per cent survival from a starting population of a million. Survival of Aspergillis niger also falls off rapidly to .03 per

cent at 100 degrees, and even some survival at 107 degrees. I have another report from the National Research Corporation on Aspergillis niger, using a combination of insults different from this. This is vacuum and ionizing radiation which are two of the conditions that we could figure as being used for the sterilization of spacecraft, either in a special sterilization facility or during the flight itself. Beginning with 1.3 million organisms and using 100,000 rad, and irradiating a sealed-off test tube containing microorganisms four to five days in the vacuum, this resulted in the recovery of 140,000 microorganisms. Using once again 100,000 rad, but placing it under a dessicator, just containing silica gel, the number dropped off very, very rapidly to 920. If one increases this rad to 200,000 plus the above vacuum, the recovery from 1,300,000 organsims is 1,000. Then they had a very strange result. If they exposed the organisms to a vacuum for five days, and then let air into the tube before irradiating it with 200,000 rads, the number dropped down to only 42 which they were able to recover.

DR. REYNOLDS: Was this dessicated air (by the way) that was allowed to come in there?

DR. QUIMBY: This is free air. Ambient air.

DR. REYNOLDS: Room air?

DR. QUIMBY: Yes.

DR. REYNOLDS: Not dry air?

DR. QUIMBY: No, this is air containing moisture. Then, if they stored the sample organisms in a dessicator for one week and exposed it to 200,000 rads, they got no recovery whatsoever. In the interests of following up on this further we also had Wilmot Castle do some similar work but

using a combination of radiation and heat. I studied a bibliography from Charles Phillips on this subject, which was somewhat inconclusive, and so we had a quick test run up at Wilmot Castle, Carl, after you left, I think.

DR. BRUCH: I set up the initial experiment.

DR. QUIMBY: These results are very interesting and then I will turn the meeting over to our chairman. Once again, using B subtilis niger and exposing the organism to 184,000 rad, just the radiation treatment itself reduced the one million organisms to 59,000. But then when they started the application of heat for one, two and three hours, quite an interesting thing developed.

DR. HOROWITZ: What temperature?

DR. QUIMBY: 120 degrees Centigrade. Starting with a million organisms, 184,000 rad reduced the population to 59,000. One hour of 120° C. resulted in a recovery of 4,000 organisms after the first hour. After the second hour, the recovery was 290. And after the third hour, the recovery was zero.

DR. BRUCH: The essential conclusions that I draw in looking at the data that they sent me is that there was no synergistic effect between dry heat and radiation.

DR. QUIMBY: I thought there was some synergism.

DR. BRUCH: It is essentially additive. In terms of D-values, in one hour you should have destroyed-reduced tenfold.

DR. QUIMBY: The reason I did not think they were additive is because normally it takes a much larger radiation than this to get this result.

DR. BRUCH: You have to realize that your radiation only knocked it down. Actually the figure should be, according to what I have here, 90,000 organisms. Then from the 90,000 you start adding your heat, and we know for this particular organism the D-value to destroy one log, 90 per cent of your population, is one hour roughly.

DR. QUIMBY: You cannot accomplish this in three hours at 140 degrees C.?

DR. BRUCH: This organism we would destroy roughly in a little over six hours.

DR. QUIMBY: I thought this one was especially resistant to heat.

DR. BRUCH: Yes, it is. But this population, D-value of one hour, would take a little over six hours to destroy with live heat.

DR. QUIMBY: Your conclusion is that they are additive and not synergistic?

DR. BRUCH: It is based on the population level after you got through with the radiation treatment, the heat did not kill any faster. In other words, with 90,000 survivors, this should take three hours of dry heat to kill. In other words, if we started with 90,000 unradiated and 90,000 radiated, it took the same amount of time to destroy the population.

DR. QUIMBY: You mean with that small population?

DR. BRUCH: Yes.

DR. QUIMBY: Dr. Phillips, this is exactly what you tried to tell me over the telephone a couple of weeks ago.

DR. CHAS. PHILLIPS: I think I said if there were a very pronounced effect, somebody should have stumbled across it by now. We have something in the literature. Some said there is a little synergistic effect,

and some think it is the opposite effect, and survivors to radiation are a little more resistant.

DR. QUIMBY: This idea of being able to insult the organism simultaneously with two different penetrating stresses at low intensities and obtaining synergism was a hope of mine. The way we stand now is that we have to heat or irradiate at such high intensities that mission reliability is jeopardized.

DR. BRUCH: Even though the effects on the organism here are strictly additive, using two sub-sterilizing treatments to get a sterile effect, you might do less damage to your components going that way.

DR. HOBBY: We do not know whether or not the same type effect would apply to the component material. Of course this we would then have to also evaluate.

DR. QUIMBY: Dr. Bruch's statement is that even though these are merely additive, it is still a worthwhile consideration because you do not have to use as much of either of them. Isn't that your point?

DR. BRUCH: Yes. Just saying the six logs, you come down three logs, 300,000 rads, and then three hours with the dry heat, and still come up with a sterile component or spacecraft.

DR. WYNNE: That is exactly what I was thinking until the chairman ruined my thoughts by saying these effects might be additive by destroying the components, too.

DR. PERKINS: On the previous study, what was the degree of vacuum?

DR. QUIMBY: 10^{-8} to 10^{-10} torr. The vacuum looks like it will do us no good at all, but the heat and ionizing radiation in combination

I think might be further explored.

DR. BRUCH: It might be the reverse effect with B subtillis niger. We were running at 100 degrees C. and I think our curves are 12 days, maybe longer than that. We have them at 110° C and 100°. We have not sent the data to you. We were doing this for Thiokol on the solid propellants and the data arrived eventually at Hughes. I know in terms of B-subtillis niger the vacuum enhances the effect of heat. With aspergillis niger, it is just the opposite, it seems. The vacuum increases the resistance of the organism.

DR. OPFELL: I would like to make another pass at the question I originally asked with respect to Item B-2 on the agenda, the reliability of single temperature heat sterilization. I would like to address the question primarily to Dr. Phillips and I would like to put it in terms of perhaps three components, because I am really quite seriously interested in how we are going to use these figures. First I would like to ask, if this ten to the minus 4 has relevance to deciding on what is usually called the fiducial probability on which you design your sample size for a specific viability; in other words, you are going to determine whether the material is sterile or not by a specific viability test. The fiducial probability determines how large the sample size will be. I made a reference earlier to the mill standard 105-A which, given the fiducial probability, you look at the sample to determine how large the sample should be. If you do this, this has applications to the costs of demonstrating this reliability, specific implications with respect to the operating characteristics of the tests, it specifies what you call the producer's hazard and the consumer's hazard.

In other words, you balance off the interests of the engineering reliability against the sterilization reliability. What I am saying is that if it is a fiducial probability, this has certain cost implications which we should certainly be able to calculate out to determine what the usefulness of this level is. The second possibility it might mean, is the spacecraft really sterile? I don't really understand how this could be a really meaningful question because a spacecraft, after it is constructed, either is or is not sterile, and it is not a probability question. We can only determine the sterility in terms of viability tests. We have never tried a viability test for all the organisms. It is a possibility, this is what it means. The third is what Dr. Price referred to, the possibility that it refers to fractional reduction of initial populations. Does it mean if we start with an initial population of say, something of the order of 10^{11}, which is referred to here, that we reduce that to a factor of 10^{4}. Is this what it really means? Or are there some other meanings?

DR. CHARLES PHILLIPS: One thing I would like to say, in our own work, in our own laboratories, we do not use the same type of testing as the pharmaceutical people. We are studying methods. We have always counted all the steps along and plotted. In other words, you have a log reduction so we can work out the same formula, the D values that they talked about, rather than just do a procedure and saying yes or no. Then usually from what plots, down to zero, this takes less testing to find out what ought to be the usual thing with such and such a procedure. Then we usually add a nice margin of safety on it. And with most of these things, either ethylene oxide with heat there is not much damage to the material so that you can add a longer time on something like that. We are not intensifying

the effect; we usually intensify the time factor which usually does not do much to the material, so that you get away with less testing. As to the second question, I don't see how we can do anything but take processes that we may know the margins of safety, and I think we can use a higher margin of safety factor than on pharmaceuticals because by and large, spacecraft are relatively rugged. Then you are coming back to something like what Jaffe did here, you are using the series of processes where each margin of safety has been considered. We test various components and I do not see why you have to test every blasted component. There are differences of bacteria encased in a solid object with nitrogen present or not on a glass cover slip, on a piece of cotton, or on a hard surface. I don't see anything we can do except take techniques which we have measured and study particularly the killing rates of the techniques.

DR. HOBBY: My impression is that in the drug industry, the tolerances are not as high as we are asking here. Is that correct?

DR. BRUCH: That is my impression.

DR. HOBBY: Nevertheless, it appears we would have to use approximately the same type of techniques in our spacecraft sterilization because we have no other alternative, it would seem.

DR. CHARLES PHILLIPS: Down at Cape Canaveral, for example, the monitoring on the ethylene oxide was done at every half-hour on the hour.

MR. HASTRUP: It is usually done every hour.

DR. CHAS. PHILLIPS: The chemical sample was taken and a relative humidity determination was made. While no effort was made to determine sterility, we did determine that the ethylene oxide did indeed stay there. We knew the relative temperature and relative humidity. We

had an hourly check of temperature,concentration and humidity.

MR. OPFELL: What you are saying is that sometimes one can go overboard in terms of sample sizes and statistical tests to verify something, where actually you can get more significant information by simple tests, or by such things as measuring viable organisms on the outlet filter with the ethylene oxide circulating system or something of that nature, a very simple thing to test, and if it fails, no matter how fancy your statistical tests may be, you simply have failed.

DR. CHAS. PHILLIPS: I would personally object, to make you feel a little happier, if we put several billion spores in there just to be sure we could run a sample. I would rather keep the whole thing clean and free of all the resistant spores, test them in the laboratory with a nice rate and determination of D values, and when you go there, try to keep it clean and don't open it up or touch it.

DR. BRUCH: You are implying we should not run biological controls? I am against that. I know in our own work with gaseous sterilization we wouldn't let anything go out of the plant unless we would run biological controls, even though we were doing relative himidity sampling and ethylene oxide. The final test that the produce was sterile was running something as Dr. Cherkin suggested. We used paper strips impregnated with spores. He used a soil sample.

DR. CHARLES PHILLIPS: To actually run the risk of adding additional contamination, you can do all kinds of biological tests except when the chips are down, and then I would like to see the thing as clean as possible.

DR. BRUCH: You could still expose it to the same deleterious agent that you are using to sterilize the capsule. If you are still in the shroud, I don't see why you couldn't put in some spore strips in the shroud and be able to retrieve them for sterility testing even though you are doing the gas analysis and the humidity analysis.

DR. OPFELL: Certainly if you have your gas re-circulating, the gas leaving the shroud on the down-stream side of the shroud should be at the same composition as that entering at steady state. So you can put all your biological controls down-stream of your shroud, and they are easily retrievable.

DR. CHARLES PHILLIPS: Of course there were a series of tests with the same shroud and temperature. The thing was contaminated and you did do biological sampling. Say you get a biological sample out of this thing. By the time you read it, the thing is very apt to be an unreliable sample.

DR. OSWALD: I am very new in this field. I want to find out if I am correct in what I have gathered so far, that the problem really boils down to penetrating sterilization rather than surface sterilization. In other words, everyone agrees that surface sterilization is relatively simple, and penetrating sterilization is very very difficult. If this be true, then we might ask ourselves the question, is deep sterilization really necessary? Is this penetrating sterilization essential? If the sterilant can't get into these parts, how is the microorganism going to get out of the part? These organisms are after all protoplasm, not ectoplasm. They can't squeeze out of a part that they have been thoroughly embedded in.

So, couldn't these non-sterilizable components be built so that they would have less than 10^{-4} possibility of rupture when they hit?

DR. QUIMBY: That is a good point, perhaps at least some of them could be so built. Also materials which would absorb some of the impact energy could be utilized where appropriate.

DR. OSWALD: Do we have to sterilize everything that the engineers bring us in this system, or can't we tell the engineers what we can sterilize and what we can't sterilize, and how thick the piece should be -- if it is a given material, how thick the piece can be that we can sterilize, and so on. And then possibly components which are not sterilizable could be made so that they would not rupture, that they wouldn't break up.

DR. QUIMBY: I think the answer to whether or not we need **deep** sterilization or only surface sterilization depends in part on whether the spacecraft does what it is supposed to do or not. If it comes in and lands on the planet at 50 feet per second on a parachute, it will not break open and the deeply-oriented contaminants will not get out. But if something goes wrong, and the capsule crash-lands on the planet, then many of the deeply-oriented organisms will break out unless, as you say, you design certain components so that they will withstand considerable impact.

DR. OSWALD: I think it should be designed for the full velocity of impact and still keep these organisms inside the little blocks of whatever they are inside of.

DR. PHILLIPS OR DR. WYNNE: The point was considered very much at one time, and there was even some talk of some experimentation. On the lunar shot, at the speed at which they are going, I don't think a bullet

would splatter, probably something like this. Solid metal would break apart. The best data we have on what the effect of some kind of a shock on microorganisms that I know of is that of withstanding a high explosive, and spores do survive being intermittently mixed with high explosives and in having the high explosives detonate. These things may be higher pressure bands and higher instantaneous temperatures on a very very hard impact landing. Actually we just don't know how hard this landing might be, whether things might break open or not. It is on the basis of that that we did the first things of cracking open and finding out whether anything lived inside the sealed objects. If nothing was living inside them, if all died off in two or three weeks after you sealed off a transistor, there was no worry. We have gone now over a year, and I think there is more data of bacteria in solid plastics. We put spores in plastic polymers and polymerized the plastic, and after a year we could sterilize the outside, open them and find living microorganisms. So they stay at least sealed imbedded in plastic for a year. I don't know if there was some decay rate, or to what extent. All these things aren't a year old for a space shot. It can be that a really hard landing might have higher pressure and shock waves than some of these figures; there was some talk of that. We just don't have data in that range.

DR. LYNCH: Would you know ahead of time, a few minutes, seconds or so, whether you were going to have a hard landing or a soft landing? In other words, could you build in the system some type of fusion device that would sterilize if something went wrong just before landing?

DR. QUIMBY: You mean a sudden in-flight sterilization or destruct?

DR. LYNCH: Just before crashing. Say, like a small atomic bomb.

MR. HASTRUP: If we lose communications, we have lost --

DR. LYNCH: And you could not destruct it.

MR. HASTRUP: We couldn't send instructions to it.

DR. QUIMBY: On Mars it would take quite a few seconds of lead time. It is at least 30 million miles. It would take quite a few minutes of lead time in order to know whether or not you were in trouble.

DR. CHERKIN: On that tack, couldn't you visualize a sensor in the capsule so that if it is coming toward a solid body at too high a rate of speed, it just sets off a hydrogen bomb or something like this and sterilize itself?

DR. LYNCH: Couldn't we sterilize just before impact if necessary?

DR. HOBBY: I don't think that would solve the problem.

DR. WYNNE: Even a signal would take three minutes to get there, or two and a half minutes.

DR. OSWALD: Rephrasing my question, can we predict, at meteoric velicities, what will and what will not survive? In other words, obviously a piece of glass would not contain any microorganisms within it because during fabrication the temperatures were up in the 16 or 17 hundreds. A piece of glass would obviously disintegrate into a piece of sand or something smaller. I can visualize polyethylene components just bouncing.

DR. OPFELL: I think in terms of the experience that your group and Dr. Price's group had in recovering below the order of, say 10^4 from

solids, that this is not a simple problem to break these up at even very high impacts, and the point that they may not come out is quite a relevant one. I think that we want to also, however, temper this encouragement from this standpoint, the question of how long will the solid remain on the Martian atmosphere and what are the conditions of these solids. It may be that while they are not liberated on impact, that after the weathering of several years in the Martian atmosphere, a variety of things would happen. These could be things that we could establish, those components which are safe and those which are not.

DR. OSWALD: This would be an intensive study of materials, their impact and weather resistance.

DR. OPFELL: A study of materials rather then organsims.

DR. OSWALD: Yes.

DR. HOBBY: These are excellent points. We have covered pretty much Item 7, the sterilization tolerance, and we have also touched on Item 2 to an extent, in discussing the heat sterilization. And also we have covered pretty much Item 3-a on the tolerances and problems. I wonder if we could get to this Item B-1 now, which is the problem of maintaining a spacecraft in a sealed condition after sterilization. In the Jaffe report there is a calculation which indicates that in order to preserve this tolerance of 10^{-4} after sterilization that the maximum permissible leakage into the shroud could be around .28, about .3 of a cc, which would be during the time after sterilization until the shroud is ejected beyond the atmosphere. It seems like an impossible engineering accomplishment to build a protective shroud with that kind of sealing capacity. I wonder if any

of you have any comments on this, or bright ideas on how we might build such a protective device.

DR. OPFELL: I have been reading an interesting book by Dr. Gumble on the statistics of streams. He addresses himself to the problem of building dams for floods. For example, the point he makes, and it is a good one, is so often we study the mean values and values of central tendency. But in cases where the outcomes are possibly catastrophic, that Dr. Phillips referred to earlier, we should be studying the statistics of extreme values. If you are looking at these shrouds, I don't think we really are concerned with the mean performances of these. We are concerned about the extreme instances, the unusual cases of human frailty in terms of remembering to put the filter in, or to put the filter in correctly. or to lock the fastener or something of this nature. It is in this area where we must identify these systematic effects that are going to come into play. It doesn't seem to be relevant to talk in terms of 10^{-4}. This implies a probability effect. These are not probability effects, these are things that are "yes" or "no" effects. It is either the thing was done correctly or it wasn't done correctly, but there is a terrific number of them. One has to establish what these possibilities are and absolutely fence them off. Any of them, if it isn't done, leads to total failure. It is like a flood over a dam of a certain size. If it is less than that, there is no problem. If it is greater than that, you have a catastrophy down the stream.

DR. HOBBY: I think what you are saying is that each part of the handling of the shroud, for example, the manipulation of putting it on and sealing it, each step has a certain probability of introducing

contamination, or say after it is on, the gassing procedure and the purge procedure, or the transporting of it from the area where the sterilization is done to the gantry, that there are probabilities at each step that you might re-introduce contamination. The final result is that after all of these things are done, you still want to end up with a net probability of 10^{-4}.

DR. OPFELL: No, that is not quite what I am saying. What I am saying is that there are a number of things that go into the operation of the shroud, transportation and so forth. If these things are done properly and the engineering is proper, then the probability of contaminating the craft from this factor is zero. If it is done improperly, you can say essentially nothing about the contamination. So the safe thing to assume is that it is contaminated with unknown amounts of organisms. So that rather than being a probabilistic phenomenon, it is one of engineering design.

MR. HASTRUP: I think that is a very good point. I would like to mention one other factor that enters in the shroud problem, and that is the aerodynamic pressures and heating that it goes through the atmosphere, under which we have no control after it is once launched. We can attempt to design it, attempt to test on the ground under simulation, but we, in fact, are not entirely realistically. There may be something that goes wrong. It may warp or do something so that the seal is broken after it has already left the Earth. So, I think this type of a catastrophic failure must be considered.

DR. OSWALD: But is there a basis on which one can make any probability statement?

MR. HASTRUP: I could mention one technique we attempt to use, and that is to maintain positive pressure inside the shroud at all times. This would tend to eliminate some factors. It still means we have the question of filters, maybe, or whatever you are using, to maintain positive pressure. Also, we have a problem in that we can't maintain this positive pressure entirely within the shroud entirely over the whole surface during ascent because the aerodynamic pressures become greater than what we can tolerate as an internal pressure. There is one other concept we might use, and that is to put an internal shroud inside the main shroud to avoid the aerodynamic effects, just around maybe the capsule that we are most concerned with. Then we have confined our problems still more and we can concentrate on a smaller area and less factors.

DR. OPFELL: This is what I am saying. It is a matter of engineering design and these are engineering steps.

DR. HOBBY: Do you have to verify that engineering design? How do you do this?

DR. OPFELL: The usual procedure is by appealing to Newton's laws of motion and data on mechanics and materials and this sort of thing. Some things have been well enough established that you just really have little need for testing purposes.

DR. HOBBY: I think there is one major problem and that is the actual seal. You have a very large cone which could be of a clam-shell configuration, which would split so that you would have an extremely large

sealing surface. The question is whether or not it is engineeringly feasible to design such a device and maintain a seal, an adequate seal over all this surface. Unless you do have positive pressure inside at all times, it seems to be quite unfeasible that you could maintain a maximum tolerance of .3 of a cc.

DR. CHARLES PHILLIPS: I didn't read three-tenths of a cc in Jaffe's paper. You mean this is the amount of air he thinks should be allowed to leak in while it is from ground level to 200,000? I think he got the data from you, I don't know whether directly or not, on how many viable organisms there are in air.

MR. WOLFE: A magic figure of ten.

DR. CHARLES PHILLIPS: Ten per cubic foot. Of course, while it is on the ground there is no problem. As long as it is holding air, anything that will keep back molecules of oxygen and nitrogen will not permit the entry of bacteria. The only thing is whether your shroud will get some air.

MR. NOWITSKY: If it is designed properly, if the right techniques are used, there is no reason why it should leak.

DR. HOBBY: How would you go about designing such a shroud?

DR. CHARLES PHILLIPS: One thing. If it does get a small crack and you do get organisms inside the shroud during that four or five minutes it is going through the atmosphere, I can't imagine they are going down into very remote cracks, or something like that. And as soon as the shroud comes off, it should get ultraviolet on it wherever the organisms impinge.

DR. QUIMBY: That is right. You would get some sterilization during flight from UV after the shroud is off.

DR. OSWALD: What is the relative pressure in this hypothetical vessel that is going out? It is going out into a diminishing pressure field. Wouldn't the tendency be for the pressure inside the vessel to be larger than the pressure outside the vessel?

MR. HASTRUP: It is at the point we are venting. The shroud is vented to reduce the pressure inside, otherwise it has to be designed so heavy to withstand the difference of pressure, and also at the time the shroud is deployed it is desired to have no pressure inside otherwise there would be a sudden release of pressure all about the spacecraft. So we are venting the shroud.

DR. OSWALD: It always keeps delta-P down --

MR. HASTRUP: Yes, it is one or two p.s.i. above, possibly, at the point we are venting. The highest aerodynamic pressures may be at the nose of the shroud. That could amount to a few p.s.i. so that we would have a higher pressure there than is present inside.

DR. OSWALD: You mean there would be a difference of pressure within this --

DR. HOBBY: At some point there would be a higher pressure outside than inside.

MR. HASTRUP: The pressure varies over the outside surface. We vent to a relatively low pressure on the outside surface, keeping the inside down, below what happens to be the maximum pressure through aerodynamic loading.

DR. HOBBY: This might occur along the seal, so that there would be a good probability of getting a leak at that point.

DR. CHERKIN: Is it feasible before launch to spray the capsule with a sterilizing fluid as you are using on mating parts?

DR. CHARLES PHILLIPS: This wouldn't stop -- if there were a leak to occur near the nose, the shrouds -- the shrouds, this one, is like a clam shell. So it does have an opening all the way around, and the way they get the capsule off is to have it open up like a shell. I think the conversation is that there is a possibility of this happening before it is at 300,000 feet or getting a slight crack. It is sealed. When it opens it breaks the seal. This is discussing something which could happen. Even though the air inside is at a higher pressure than outside, if the crack were at the tip of the nose, the outside pressures could push a little air in possibly. I don't know how likely it is.

MR. WOLFE: If there is something going to happen to the pressure, pressure-sensing devices are very highly evolved. There are a lot of excellent things in that line. Certainly you can push the destruct button if something like that happens.

MR. HASTRUP: We are talking about something with a huge volume, and the amount of leakage that is being considered is very very small, undetectable by any present pressure devices.

DR. PRICE: I think what we are looking for is a self-sterilizing seal, based upon our knowledge of permeability of various gases through polyvinyl chloride, and so forth. It is conceivable we could sterilize through the seal. I don't have the engineering details worked out, but perhaps we could use these permeation rates to our advantage so that we could not only sterilize the mating surfaces but have the seal in there which would permit gases to be vented and based upon molecular sizes allow

nitrogen to issue forth and not permit microorganisms to enter.

MR. HASTRUP: You are talking about a filter?

DR. PRICE: A filter that would be in effect a seal for us.

MR. HASTRUP: This has been suggested, that we use essentially a gasket around as a seal, or as a back-up seal, so that if there was an actual leak, that the leakage would have to pass through a bacteriological filter.

DR. QUIMBY: What kind of seal do you have now?

DR. PRICE: Metal to metal.

DR. CHARLES PHILLIPS: There is one other relatively simple thing that could be done, engineering-wise. Some of the best bacteriological samplers work on the principle that air goes around a corner real fast. Bacteria have weight and momentum. We can have a hundred percent efficient filter merely by putting a stream of air against a metal plate, while the air goes around the plate the bacteria will all impinge. So that if there were a lip in the inside of that thing, even if a little bit of air leaked out. all it would do is impinge on the top part of that lip and stick there. Then when the thing was opened and the shroud thrown away, it still wouldn't have gone in. In other words, if the shroud is so constructed that even if it opened a little bit and let a little bit of air in, that air had to then make about a 90 degree turn, it would clean itself in so doing.

DR. HOBBY: You could impact it on a sticky surface maybe.

DR. CHARLES PHILLIPS: No, you don't need a sticky surface. Just metal will do it. And there are all kinds of bacteriological samplers that are based on this principle.

MR. HASTRUP: Of course the pressure of the vessel must be controlled. It may be that these wouldn't work over the wide range of velocity that could occur through the leak.

DR. CHARLES PHILLIPS: The mechanics of this, the theory of impingement of small particles from aerosol, are very, very carefully worked out. It would be very easy to work out what you would have at certain leaks. I imagine if there is any overlap at all so that air would go in, and impinge, you would catch it in the overlap. Frankly, I don't think there is an extremely serious likelihood of contamination on the way up. I think the mechanical factors are the factor that with a little look at the shroud design so that we might know where the air jets might go, where the microorganisms might go if there is a leak, will do it. If a hunk of the shroud goes, you have probably aborted the flight anyhow.

MR. WOLFE: I don't know how practical an idea this is: Can you keep the shroud on until you get through the Van Allen belts?

MR. HASTRUP: It is desirable to remove it as soon as possible to save payload weight. They usually leave it on long enough to get above the high aerodynamic loading. This is what has been determined. It happens to be high enough so that we feel it is above the contamination level in the atmosphere.

DR. CHERKIN: How hot does that shroud get?

MR. HASTRUP: I don't have any figures.

DR. CHERKIN: Is it metal?

MR. HASTRUP: On the Ranger it is metal, largely metal. On the Centaur launch vehicle it would be plastic, fiberglass construction, by and large. This is partly to serve as an insulator because the heat

loading is fairly high. They like to keep the inside temperature down so there wouldn't be any smoking or burning of inside material.

DR. PRICE: Getting back to an earlier comment of Dr. Phillips of the hospital sterilization nurse, one thing we hit upon in quizzing personnel working in cleaning room procedures, and those individuals who would be directly responsible for maintaining the degree of cleanliness adequate, was an awareness that at any time there could be a relaxation of the sterility characteristic based upon a last-minute decision to go ahead even though it is not quite sterile. We expressed one hope, that if they bestowed the responsibility of maintaining sterility, they would like an assurance that there would be no relaxing of this so that from a human factors point of view they want us to have a solid front and have no possible exceptions made. This would introduce the problem of the very important aspect of the human factors of education and integration problems that the whole sterilant program brings to bear in a launch.

DR. QUIMBY: Organization, policing, control, and authority.

DR. PRICE: Yes. It is extremely important. People concerned have this fear.

DR. OSWALD: What if this shroud were pressurized with ten percent ethylene oxide in CO_2?

MR. HASTRUP: It only takes a few minutes to get up to where it is deployed.

DR. OSWALD: I am only thinking about the last minute pressurization to make sure it doesn't become contaminated at the last moment, at the time of separation, or something like that.

MR. HASTRUP: I thought you were thinking that by introducing ethylene oxide I may sterilize anything that came in. Is that what you have in mind, that you would prevent recontamination by sterilizing with the ethylene oxide?

DR. OSWALD: This takes too long.

MR. HASTRUP: Yes, this process takes a matter of hours.

DR. OSWALD: We are assuming that we have a sterile unit to begin with, and you are simply maintaining sterility up through the atmosphere.

DR. HOBBY: That is what we are trying to do.

DR. CHARLES PHILLIPS: If I understand the question, the point you raise, ethylene oxide wouldn't be rapid acting enough. This is only a matter of a couple of minutes and the inside atmosphere is all being rapidly lost anyhow, you see. The concentration is dropping as fast as it can. You are losing it. The engineers don't like ethylene oxide for another reason, particularly when they are using it with freon. It weighs more. It adds quite a few pounds weight. They would like to replace it at last with a gas; air, for example, or nitrogen, that is considerably lighter in weight, and you gain several pounds by taking the ethylene oxide out.

DR. OPFELL: I think the substance of the discussion here, when we originally raised the question, was the probability of sealing in the capsule. I made the contention that this is primarily an engineering problem or can be solved by engineering. I think this has been pretty much confirmed by the discussion. I think Dr. Price has made a very important point in the area in which it should be emphasized, namely, in

the aspect of industrial engineering. His pointing this out reminds me in terms of the pharmaceutical industry, that is where we put our first effort.

DR. PRICE: We took a hypothetical vehicle and moved through operations leading up to the launch site. All the way along the way the observations made confirm your earlier comment. There was either a maintenance of sterility or a complete break. We couldn't ask about the contamination that had occurred. When we began examining the breaks, they were all severe. In the loading platforms, before they were shipped at the Cape, and at the Cape, contamination levels were extremely high, higher than one could attempt to ascertain.

DR. HOROWITZ: It is still a probabilistic problem in the sense if you carried out these same procedures a million times you would have a few instances where you got contamination, even though everything worked apparently perfectly. There is no real ultimate sterilization in this sense. I have been bothered ever since the comment Dr. Opfell made about an hour ago; he said he didn't know what 10^{-4} meant. I think he should explain this. If you start with a load of 10^{11} organisms does it mean you are reducing them by a factor of 10^{-4}, in other words to 10^{7}, or does it mean you are reducing it by a factor of 10^{15}, to 10^{-4}? My interpretation, the latter is correct.

DR. HOBBY: I think I did make that statement.

DR. HOROWITZ: Does it give you a kill so there is only one survivor out of the population of 10^{15}?

DR. OPFELL: I hope the rule is that this refers not to a priori probability but to a posteriori probability. In other words,

trying to decide from empirical data what the orders are. This we do in terms of defining confidence limits on what the true situation is from measurements.

DR. HOROWITZ: If you have mortality curves you can predict.

DR. OPFELL: We don't want any number there. We don't want to end up with 10 or 15 but zero. We can afford to spend only so much money to establish this. Beyond this we can't do it. We used this number in order to decide what it is going to cost us to get a certain level of confidence on the zero contamination.

DR. HOROWITZ: You never can end up with zero. Organisms die exponentially.

MR. NOWITSKY: You are not sure of any of the mission parts. You just do the best you can.

DR. HOROWITZ: This is the goal. Nobody wants more than one contaminated payload out of 10^4.

DR. CHAS. PHILLIPS: I read a different thing into his report. He looked at what we were doing and can do and figured it came to about 10^{-4}. Then he asked, would we buy this, would our current procedures give us 10^{-4}. This is as I read his document.

DR. HOBBY: I don't think that is true.

DR. BRUCH: I don't think it is either.

MR. HASTRUP: I thought he arrived at a number on a scientific basis of what we would like to achieve biologically, and I think that he would believe that if it was necessary to have 10^{-6}, that this could be achieved by a simple means of heating as he actually proposes for achieving 10^{-4}. I think he tried to arrive at the number initially on

what we would like to have as a goal, the lowest possible goal that we would accept, and what procedures could be utilized to achieve that goal.

DR. CHAS. PHILLIPS: I thought he ruled out complete heating.

MR. HASTRUP: He recommends it.

DR. CHAS. PHILLIPS: Does he think he can get it with the first Mariner shot?

MR. HASTRUP: He does leave a loophole, that you can possibly get by with glove-box assembly in case we do have a few exceptions in the early missions.

DR. HOBBY: The recommendation was that we use total heat sterilization, and that if there were any exceptions, in order to achieve these tolerances we would have to use a dry-box procedure, using ethylene oxide. One step ethylene oxide processes. What we mean by that, you put the things to be assembled in a dry box, unassembled, you sterilize with ethylene oxide, purge the box, and then proceed to do the assembly. He did not consider the use of liquid sterilants at all satisfactory for this. So actually the only alternative was the dry-box procedure using ethylene oxide.

Second, as a development engineer I would like to make a comment. It appears that what you are trying to do here is establish the highest level that you can achieve. It may be that by establishing a level of 10^{-4}, for instance, you, as a matter of fact, achieve a much lower level in practice than you would by establishing a lower level as a goal initially. As a typical "for instance" of this, let's talk about the Thiokol retrorocket where the goal was to try and achieve 10^{-4} and the only method of achieving this, -- and there are many components like this --

was terminal sterilization. An attempt then was made to develop a component in which this was to be the primary mechanism. It came out that it was impossible to sterilize this by this technique and so you have no sterilization period. You solve the sterilization problem by a waiver. Let's say -- yes, that is exactly what happened -- let's say that 10^{-4} is established, and on the basis of this 10^{-4} say okay, the only way we can achieve this is terminal sterilization. And so you go down to the line saying this is the way we are going to do it, we are going to achieve this level. You then get down to the Cape and you find out that there is a transistor, a squib, a diode, which cannot be thermally sterilized. You now have a choice, you either do not fly or you do not thermally sterilize. If you fly, you fly with the spacecraft whose contamination level is much, much higher than you might have achieved if you had said 10^{-1} or 10^{-2}. Recognize that there may be some things like this and say that we will accept a lower level and we will accept some of this glove-box technique. As a practical development problem we have seen on the lunar program that we are ending up with non-sterilized lunar spacecraft, and there is no question about it. We are not sterilizing. We have not sterilized the Rangers; we are not going to sterilize the Surveyors. This is fact. Whether or not we sterilize the Mariners, or what level of sterilization we achieve, this to a great extent may well be on the basis of the level that you people establish. If you establish 10^{-4}, you may get nothing. I think it is worth considering. My point is that if we aim at lower tolerances we may get still lower tolerances. It seems to me you always shoot higher if you expect a hit.

MR. PIASECKI: Except by shooting higher it may make you miss the target completely. You must recognize that there is another probability in shooting very high, that you may go to a mode that you now have to insist upon, or you get nothing. This is the only point I am making. There is no question, of course, that in everybody's mind we want to achieve as high a level of sterility as is possible with all the practical constraints that are in here. But the concern is that you may end up by establishing a very high level of sterilization. The implications of this in a practical sense may be that you have to go to thermal sterilization after all. Then you may find out, late, as a typical "for instance" that perhaps we should have put inputs into the designers, that they make the components thinner so that the heat capacities are smaller. The Mariner-B capsule is on the design table now. In other words, the inputs that you should have put into the designer should have been based on two years of work which should have been done two years ago. It is too late to put these inputs in. You are going to have to take what goes. The safety people at the Cape have the same problem. They complain because the squibs are much lower-powered squibs and it is unfortunate. But those squibs were designed two years previous to when they established their requirements. You simply can't make these kinds of changes.

DR. QUIMBY: I don't think it is too late. I think the Mariner-B capsule and its components can be looked at one by one. I think we have lots of time on Mariner-B. Perhaps up to 1966.

MR. PIASECKI: We may have if there are some changes in schedule.

DR. QUIMBY: That's exactly what I mean, so far as the original Mariner B is concerned.

DR. REYNOLDS: There are one or two points I would like to make. I think the prime impetus for our calling this meeting -- I am not sure that I made this sufficiently clear at the beginning, although maybe it was covered in the letter -- was that the Space Sciences Steering Committee was asked to react to these proposed guidelines which you have copies of. This further was to be passed on to the Biosciences Subcommittee which isn't really fully formed yet, and besides would not have had the kind of special knowledge that would be required really to go over this thing in the way that we have been going over it today. One of the things that we ought to be sure to come up with today, before we get through -- and that is the reason I am emphasizing it now -- is a pretty clear idea of the extent to which we want to concur with these guidelines.

The remainder of the conference was devoted to the revision of Mr. Jaffe's guidelines. These have in turn been revised by the Iowa City OSS Space Science Summer Study. The final guidelines are now as follows:

1. For Mariner buses and booster last-stages, either sterilization must be used, or trajectories must be controlled to insure not over 10^{-4} probability of hitting Mars and not over 10^{-2} probability of hitting Venus.

2. A Mariner entry capsule for Mars should be given recognized and accepted (official) sterilization treatment and handled aseptically thereafter. The goal of these activities should be that there is less than 10^{-4} probability that a single living organism is released on the planet's surface.

This figure takes into account the probabilities of sterilization during Mars entry and impact and of releasing organisms from the capsule at the planet.

3. To achieve this probability, capsule sealing and separation mechanisms must be designed to provide a very high degree of assurance that leaks and malfunctions which would infect the capsule cannot occur prior to or during launch or at capsule separation.

4. Capsule sterilization should, if possible, be by heat in the final sealed container, with no access permitted or mechanically possible thereafter.

5. If heat sterilization of the entire capsule is impossible, heat sterilization should be used on as large an assembly as possible, and sterile parts, including fluids, added by a glove box procedure using ethylene oxide in the box. All packages, components, materials, fluids, and tools must be sterilized. Cognizant engineers should be held responsible for sterility to be achieved and maintained according to procedures specified and supervised by a Control Sterility Group.

6. A Control Sterility Group should be established as a unit within the organization responsible for planetary missions. This unit should be invested with authority appropriate for meeting the following responsibilities:

a. Designation of certified (official) procedures to be followed in order to achieve and to maintain the sterility of space probes.

b. Instruction and training of cognizant engineers in specific procedures designated by the C.S.G.

c. Continuous monitoring and recording of sterilization procedures and sterility maintenance.

d. Reporting progress and results of sterility program directly to Project Management and to NASA headquarters.

e. Recommending research and development work to improve sterility procedures.

7. Assembly, disassembly, repair, or calibration operations on a sterilized capsule should be permitted only under rigid sterility control.

8. Contamination of the moon does not constitute as serious a problem as in the case of the planets. Nevertheless, lunar contamination should be kept at a feasible minimum. For this reason the lunar probe sterilization program should continue in order to insure that the contamination of the moon will remain below that which will seriously interfere with future biological and chemical surveys, and also to advance the art of space probe sterilization.

9. A spacecraft sterilization handbook should be assembled that would include the following:

a. Complete directions for effective techniques of sterilizing spacecraft components, subsystems and assemblies. The various techniques should provide for:

(1) Internal Sterilization
(2) Aseptic assembly and applicability of clean room facilities
(3) Terminal Sterilization
(4) Other methods to include emergency provisions

b. Standard operational procedures for the determination of levels of contamination.

c. An enumeration of key organisms, test material methods to be used for sterility testing.

d. Methods for the preservation and monitoring of sterility.

Appendices would include:

1. Toxicities
2. Temperature vs. Time Curves
3. Physiochemical properties of sterilization agents
4. Handling procedures and provisions
5. Qualified components list

RECOMMENDED AREAS FOR RESEARCH AND DEVELOPMENT

Dr. Carl W. Bruch

1. Determination of levels and types of microbial contamination on surfaces of fully assembled spacecraft.

   A. The purpose of this survey would be to reduce the time - temperature cycles based now on sterilization of small soil samples.

   B. A corollary project would be the determination of microbial spores trapped within solids. On the basis of my studies with dry heat sterilization, I predict that the assay of organisms trapped in solids will prove to be an almost insurmountable task. The chief difficulty will be the reduction of a solid to a particle size that will allow the outgrowth of individual spores.

   C. It is my opinion that the level of spores entrapped in solids is so low that dry heat cycles that will sterilize the outer and inner surfaces of assembled probes will kill entrapped microbes.

2. Further confirmation of the D values for spores of known microorganisms.

   A. Additional research on the biological factors (media on which spores are grown, amount of bound water, presence of various cations and dipicolinic acid in spores, recovery media, etc.) that influence dry heat resistance of spores.

   B. Additional research on chemical and physical factors (type of dry heat, amount of moisture in gaseous environment, stability of gaseous environments around spores, composition of gaseous environments, etc.) that affect the dry heat sterilization process and spore resistance.

3. Since it is this writer's opinion that dry heat will be the only agent available for terminal sterilization of a probe, heat penetration studies should be conducted on assembled probes.

4. After these heat penetration studies are completed, a probe should be deliberately surface contaminated with a known number of dry-heat resistant spores, given a sterilization cycle based on the D values for the contaminating organism, and then be checked for sterility by disassembly within a sterile hood system.

5. Research on liquid sporicides for mating surfaces and for addition to monomers before polymerization.

6. Research on the sterile assembly of heat sensitive components or subassemblies in glove boxes.

   A. A program of adequate sterility testing must be set up to prove that such procedures will yield a sterile product.

7. Development of adequate shrouds or in-space surface heating methods to preserve sterility of launched probes during exit from earth.

8. Determination of the ability of microbial spores to grow and reproduce under simulated Martian environments

A. If it can be shown that no sporeformer can grow under Martian conditions as deduced from latest scientific data, then an additional reduction in the probability of an extraterrestrial infection would be available.

Dr. Arthur Cherkin

If the biochemical correlates of thermal and chemical death in bacterial spores are not accurately known, they should be explored. Presumably, there is a rate-determining chemical reaction, the kinetics of which should resemble the known kinetics of thermal and chemical death. Possibly techniques could be devised for large-scale separation of just-dead spores from viable spores, after short periods of heating or chemical treatment, so that biochemical changes in the dead spores can be studied before excessive degradation has occurred.

Study of the nature of the influence of contact surfaces upon spore survival is needed. Why are spores on soil more resistant than the same spores on glass? Can we infer that thermal and chemical killing involves a reaction at the surface of the spore? If not, then why does the nature of the contact surface affect spore survival?

I second the suggestion mentioned by Dr. Vishniac in Iowa City, that the first lunar and planetary probes should carry sampling devices which can take a sample of the surface matter and seal it off as an authentic, uncontaminated, original sample. It would be desirable to have at least ten such sampling devices striking the moon, Venus, and Mars, as soon as this can be done. Obviously, such devices must have some means for signalling their location, so that they can be found later.

It may be well to study the feasibility of applying a lightweight external "cushion" to all components which cannot be sterilized internally, in order to prevent rupture of the components and liberation of entrapped spores during a hard landing.

One of my colleagues, Mr. John G. Hainsworth, suggests that it might be worthwhile to re-examine the use of silver compounds adsorbed on various materials, for sterilization of liquids and possibly for self-sterilizing surfaces. Considerable work was done with silver sterilants for water sanitation at Fort Detrick, about 1945-1946. I understand that Professor Alexander Goetz of the Department of Physics, California Institute of Technology, has considerable information on this type of sterilization.

Between now and planetary launch time, experiments involving all kinds of materials may be proposed. It would be well to have an array of sterilization procedures available, so that an important experiment would not be rejected simply for lack of a suitable sterilization process. Further study should be made of designing spacecraft to have an insert of sufficient size to hold a potential new experiment, not conceived of today. The insert should permit easy aseptic assembly into the spacecraft.

Unless a good system of positive controls is already in use, I suggest that one be developed, to include: fail-safe procedure for introducing parts with known contamination; and retrieval of contaminated specimens which may slip by the normal control system. (Here, I do not have in mind protection against sabotage, but only against deviations which the normal controls are supposed to detect.)

As a coincidence, the "Research Grants Index, Fiscal Year 1961," of the Department of Health, Education, and Welfare has just reached me. Some of the projects supported by HEW appear to relate to your sterilization interests, and I therefore attach a summary of them, for your convenience.

Dr. Norman H. Horowitz

Aside from the obvious goal of finding sterilization treatments compatible with all spacecraft components, I have only one specific proposal for research in the sterilization area. This is to determine the viability of various microorganisms under the temperature regime which obtains on Mars. I realize that this is already being done by Armour, but I am not altogether satisfied with what I have seen of their results. What I would like to see is a thoroughgoing appraisal of the ability of a selected group of organisms to survive when subjected to a diurnal freeze-thaw cycle such as occurs at all Martian latitudes except those where the temperature is below freezing at all times. What I think we might find is that organisms subjected to this treatment will not survive if they are in a medium in which growth can be initiated during the thaw phase of the cycle. Cells which are not in such a medium might survive without multiplying. It might also be predicted that cells which are in an extremely favorable medium might multiply enough during the period of thaw to offset the mortality due to the freezing-and-thawing. This last point is the one about which there is the most uncertainty. It is also the point of greatest interest from a biological point-of-view, because it is only net multiplication of organisms that is of concern when we talk about contaminating the planets. Organisms which merely survive are a negligible hazard.

In other words, I am proposing that Mars may be less hospitable to terrestrial contaminants than we imagine it is, owing to the freeze-thaw cycle. I would like to see this tested experimentally.

Mr. Rolf C. Hastrup

1. Dry Heat Sterilization

a. More laboratory work should be done to establish a sound basis for the dry heat sterilization requirement. It is not believed that the work indicated in the latest Wilmot Castle work statement is sufficient. The Wilmot Castle studies are limited to the technique of end point determination for soils and other environments which enhance the resistance of bacteria. More complete quantitative log survival data should be obtained for soils and other environments in order to establish a more accurate thermal death time curve than is possible by the present end point determination technique. This may require separation of the organism from the soils and re-inoculation onto sterile soil to improve control over the experiment.

b. It would also be helpful to understanding the problem and designing tests if the soil constituents could be segregated and tested separately or in limited combinations to determine the effect on bacteria resistance. Ultimately, such testing may lead to some understanding of the basic mechanism(s) of dry heat sterilization which could prove useful in establishing procedures.

c. It may be possible that a combination of heat and chemical action would be useful for sterilizing certain items which could not tolerate the standard dry heat sterilization cycle. For example, it may be possible to sterilize solid propellants at a lower temperature cycle if suitable chemicals are present which will assist the accomplishment of sterilization. This effort should be carried on in close coordination with the development of components which are found very difficult or impossible to sterilize by the standard dry heat cycle.

d. It is also possible that other physical adjuvants could be used to advantage in conjunction with the dry heat sterilization cycle. Dynamic Science has recently submitted a proposal suggesting the possibility that temperature cycling prior to heat sterilization, which would cause freezing of the spore, may cause sufficient physical change in organisms to reduce their resistance to dry heat sterilization. It may be worthwhile to conduct a few tests on the resistant soil organisms to determine if a significant effect could be obtained by this technique.

2. Ethylene Oxide Sterilization

The present ethylene oxide sterilization procedure is based, essentially, on the results of tests with a single strain of bacteria in an isolated state. Some recent tests indicate other strains, as they exist in the natural environment of dust, are more resistant to the present ethylene oxide treatment. Controlled tests are required to determine the effectiveness of the ETO method of sterilization for various resistant strains of bacteria, including organisms existing in soil, dust, and other representative spacecraft contamination. Tests of the ETO sterilization process

should be conducted as a function of the various important variables for the purpose of optimizing the sterilization capability of this process and determining the maximum levels of contamination capable of being sterilized within reasonable time periods. Some of the important variables which should be considered are: gas composition (especially ETO concentration and relative humidity), temperature, substrate materials which may provide protective effect, previous conditioning of organisms such as exposure in space simulator, method of supplying ETO mixture (e.g., with or without initial evacuation), hardware configuration as it would influence gas diffusion, penetration of ETO for sterilization of films, grease, thermal insulation materials, resin coatings, etc.

Mr. Harold W. Wolf

Perhaps before I offer some suggestions, I might remind you that Russia may very well set the standards in respect to sterilization of space vehicles since it would be silly for us to be carefully cleansed only to have to trod in Russia's dirty footsteps. Seriously, every possible effort should be made to communicate with the Russians on this subject.

A most obvious area of research is that of the sterilization of materials. I concur with Charlie Phillips on the need of a sterility handbook. The most important part would be the list of materials and the effects of sterilization processes thereon. This should also mention the detrimental effects--not only the successful ways.

An area of developmental research is posed by that of the assemblies themselves. In all sterility work, controls are essential. Since shapes, matings of different materials, and their locations in the unit are of prime importance, the sterility group should be provided with a duplicate assembly for study and control purposes.

N.A.S.A. should maintain laboratories that can recreate the environmental conditions of any heavenly body, i.e. satellite or planet, or of space itself, whereby earth biota can be studied under varying conditions. It appears most primising to utilize solar heat as a means of sterilizing space vehicles.

The problem of how to sample and count organisms is still far from being simple. Certainly, studies in this difficult area can always be of value.

A last suggestion I have to make is that of determining the feasibility of excluding airborne microflora from entry through leaks by means of engineering design. In fact, engineering design with considerations of contamination control would be of value in the design of the entire space craft.

Mr. Robert J. Varga

Very basic to any sterilization program and the most important factor for a contractor is the definition of an acceptable method of determining sterility. At present the proof of compliance to contractual requirements is still undefined. Hence work in this area appears mandatory. To quantitatively analyze for absolute sterility is not within the state of the art; however, methods must be established to the best of current technology to establish what method would act as an indicator.

Two years ago Hughes, on their own initiative, pursued research studies to investigate the viability of various organisms to hard vacuums. Undue publicity had been afforded our findings which related that only four types of microorganisms exposed to a vacuum of 6×10^{-9} Torr for 30 days had been rendered inviable. These tests can by no means indicate that all organisms will be killed by hard vacuum; however, continued work in this area seems most pertinent. In addition, other space environments should be considered, i.e., ultraviolet light and cosmic radiation.

In conjunction with discussions with Dr. Bruch, formerly of Wilmot Castle, I know that certain studies are under way for determining adequate heat exposure schedules to adequately sterilize items with heat and other methods. Once schedules are ascertained, an evaluation of components and materials is provided through compatibility studies. However, little information is provided of a reliability nature. Hence the accuracy of reliability predictions is severely hampered. To overcome this deficiency, it is recommended that a test program be pursued evaluating the effects of each method of sterilization on reliability. Summarizing the program would consist of:

a. Selection of sufficient sample sizes of generic components used on a particular spacecraft and divide in groups according to the number of methods of sterilization.

b. Test each group for functional performance.

c. Subject each group to a sterilization process.

d. Test for performance again.

e. Subject each group to a final test under expected mission time and environment.

Compare $\bar{x}$, σ, of test results by student's "t" (comparative difference tests) test for magnitude of effect among sterilization processes. Performance of the above tests would permit analysis for the effect of any specific sterilization process on reliability for any intended mission.

Continued efforts should be expended in the development of liquid and grease sterilants because of the necessity of testing, assembling, fueling and pressurizing equipment after the major sterilization processes have been performed. Adequate liquid sterilants are yet to be firmly established. To increase the probability of launching a vehicle without failed parts, abbreviated tests are desirable. Limited breaking and mating of electrical connections require liquid sterilants. To actuate mechanisms for prelaunch checkout, grease sterilants are a necessity and should be developed.

The development of sterilizing paints, which could be sprayed on a vehicle, could ease problems of bacterial fallout. The paint would maintain its sterilizing action as long as it is in contact with the spacecraft. The paint could be developed to sublime in space or have properties satisfactory for thermal surface requirements as needed. In the problem of painted surface defacements, sterilizing paints can be developed for retouching.

In the area of solid rocket propellant sterilization, a separate development program appears advisable. Sterilizing additives to the propellants appears feasible; however, sufficient emphasis has not been allotted to this investigation. A combination of low level heat and radiation or even a combination of heat or radiation with a bacteriocidal additive could effectively render a propellant sterile.

To alleviate count-down procedures at AMR, in-flight terminal gas sterilization should be investigated. It is possible to fill the shroud at a predetermined time prior to launch and left there to decontaminate during launch until the shroud is deployed in transit. A sacrifice to payload would be incurred but a trade-off of schedule risk would be required.

An avenue for further study, with respect to sterilization, is the field of ultrasonics. High kill rates have been obtained utilizing the ultrasonic generator. Many papers and scientific journals have followed the progress of this phenomenon. Recently the application of ultrasonics to the field of medicine has focused new attention on ultrasonic sterilization. Further study may prove this field to be a feasible method of sterilization in some cases.

It should be mentioned that deviations from the standard methods of sterilization have not been considered. These methods include heat (24 hours at 257^{o}F) and ETO gas sterilization processes. It is presumed that tremendous amounts of work have been done along these lines and these methods prove most desirable.

Dr. William J. Oswald

1. Methods of producing components which will be internally sterile.

2. Develop a complete list of space-craft materials followed by laboratory studies of the impact resistance of these component materials to determine which will disintegrate on Lunar, Mars or Venus impact and which will not. Presumably non-shatterable components only need be surface sterilized, hence need be neither produced with aseptic techniques nor heat sterilized. We have some definite ideas for research in this area.

3. Develop plastics which are useful for space-craft components and which will not shatter on impact.

4. Develop special methods and materials for sterilization of delicate or sensitive electrical equipment which will not tolerate heat sterilization.

5. Determine the "lapse rate" for numbers or bacteria and bacterial species in the earth's atmosphere.

6. Develop remote control assembly procedures.

7. Study the basic biochemical reactions which underlie sterilization of microbes and viruses.

Dr. John B. Opfell

The subcellular molecular effects of exposure to elevated temperatures in bacterial spores should be studied in order to develop a theoretical basis for extrapolating thermal sterilization process and to develop rational procedures for disinfecting and sterilizing spacecraft components as well as specimens returned from other planets. Empirical evidence on the biological properties of a few forms of earth life is hardly sufficient basis for designing a process to control potential infectious agents brought by returning spacecraft.

The subcellular molecular effects of exposure of spores to the so-called "alkylating agents" should be studied to determine why they are so effective as sterilants and why so many of them are potentially carcinogenic. There exists the distinct possibility that some organisms may be stimulated to unusually rapid proliferation by contact with these agents. The precise biochemistry and subcellular energy transfer mechanisms associated with the use of these compounds should be established.

Methods for reliable testing of solids for viable cell content should be developed. Some work is now in progress on this subject. The information will be necessary if sterility tests on solids are ever to approach the

reliability now associated with sterility tests on liquids.

The development of rapid methods of gas sterilization will require further study of the physics and the chemistry of the interaction between the sterilants and the bacterial cell walls and protoplasm. Certain chemicals can pass through the cell wall readily, others cannot. The kinetics of the sterilization process on a chemical level should be established in order to determine the ultimate usefulness of engineering sterilization processes based on gas sterilization.

The kinetics of the reactions between various sterilizing agents and the substrates which they are expected to sterilize should be established. The chemistry of ethylene oxide, for example, is well developed in certain areas, in others, it is almost a complete unknown. The properties of ethylene oxide which are of significance bacteriologically can be established and should be established in the same way as those which are of significance fuelwise. These chemical studies really should precede compatibility studies with materials. The compatibility studies have been to a large degree, screening analyses with little quantitative information produced. Undoubtedly, each of these sterilants will be destructive to materials of interest under certain, yet unknown, conditions of temperature, composition, and length of exposure. Kinetic studies will permit better design of compatibility studies, yielding ultimately more reliable information for less effort.

Dr. Richard W. Price

1. The development of a concise sterilization handbook that would gather the abundant pertinent information and present useful guidelines to concerned individuals and agencies.

2. The design and testing of a training program to study the most effective means for preparing personnel in the techniques of assembling a sterile spacecraft and methods to insure the launching of a sterile payload.

3. A compilation of guidelines to govern the construction of assembly hangars, explosive safety areas, and gastry facilities at the AMR and PMR launch sites to insure the launching of sterile spacecraft.

4. Studies of the genetic effects of sublethal sterilization procedures on terrestrial microorganisms to attempt interpretation of such results in studies of extraterrestrial organisms.

5. To study the mutagenic effects of chemical sterilants on organisms planned for use in the closed ecological systems for space stations.

6. To study the applicability of existing clean-room facilities for preparation of sterile spacecraft components and subassemblies.

7. To conduct additional studies on the toxicological problems resulting from the prolonged use of chemical sterilants on personnel.

8. The development of a model sterilization facility that would provide for the stringent requirements of spacecraft intended for planetary impact.

9. To determine the long-term effects of chemical sterilants on spacecraft construction materials under simulated space, Martian and Venusian conditions.

10. To identify and characterize the most troublesome contaminating microorganisms of various geographic locations and characteristic to industries which contribute to the spacecraft assembly.

11. To study techniques to permit the shortening of sterilization procedures by chemical and physical means. Fragmentary information suggests that certain catalysts can be used with ethylene dioxide to hasten the oxidation of microorganisms.

12. To study chemical contaminants resulting from the landing of spacecraft on a planet in order to produce a molecular inventory that would be used in the evaluation of extraterrestrial atmospheres.

13. To study the most effective procedures for the sterilization of returning samples of lunar and planetary "stuff" if pathogens are identified.

14. The identification of constituents for the media to best assess sterility of space-oriented hardware. These media would further serve to expand the studies for the detection of extraterrestrial life.

Mr. Daniel N. Tompkins

1. Establish a method by which Ordnance devices, i.e., squibs, rocket motor propellants, etc. may be rendered biologically sterile.

2. Chemical and mechanical compatibility of materials with

   a) Resins
   b) E.T.O. (ethylene oxide - freon mixture)
   c) Liquid surface sterilants

3. Establish an acceptable procedure by which non-solid assemblies may be adequately gas sterilized (e.g., laminations, honeycomb).

4. Compile a list of quality assurance acceptable sporicidal resin systems. This involves

   a) Biological tests (2 types: bulk and surface)

   b) Life, i.e., shelf

c) Mechanical materials testing

d) Generate appropriate specifications

5. Establishment of valid biological testing techniques.

6. Establishment of "foolproof" sterile assembly processes.

7. Establish validity of sterilization through vacuum exposure.

8. Determine effects of radiation dosage upon resin systems.

9. Development of critical components such as batteries which will be operable after heat sterilization.

Dr. Victoria Lynch

1. A research program to determine a suitable test organism for sterilization experimentation. This program should be divided into two parts and be conducted simultaneously.

Part A: A program using suitable enrichment techniques to find those organisms most resistant to various sterilization procedures.

Part B: A systematic investigation of those organisms and test samples currently being used in the testing of sterilization procedures.

Successful completions of Part A would increase the assurance of sterility and Part B would assist in the interpretation and comparison of data obtained in the multitude of laboratories now conducting sterilization experiments with a variety of test organisms.

2. A research proposal to discover materials that are self-sterilizing. Use of these self-sterilizing materials in manufacturing processes would automatically result in sterile products under normal production procedures.

3. A systematic investigation of compatibility of materials with methods of sterilization should be undertaken. This program should establish acceptable sterilizing procedures for each material and component to be used in lunar or planetary landing vehicles.

4. A development program to establish operating procedures to fabricate sterile sub-assemblies, assemblies and completed vehicles is necessary. This should include detailed investigations of establishing a truly sterile room and the development of monitoring systems to determine that the sterile room remains sterile during assembly operation. Industrial white rooms and hospital operating rooms are steps in the right direction. However, these rooms are not sterile rooms.

As a result of this program, those individuals designing and fabricating a space vehicle will have the necessary information to sterilize initial components and the procedures to assemble a sterile vehicle.

For a Mars trip no deviation from these principles and techniques should be tolerated. Mars should be declared a biological presence and every effort be made to maintain its natural state until all biological investigations have been completed. A condition of absolute sterility must be made a prerequisite of each Mars flight. Just as a broken electrical connection is reason for a flight hold, a broken sterility step must also be a reason for a flight hold. A non-sterile craft is a non-flyable craft.

Mr. G. Briggs Phillips

It is my impression from attendance at the Ad Hoc Sterilization Conference on July 9, 1962 that several R and D projects on heat and gaseous sterilization procedures and techniques are presently being supported by NASA. I assume that these projects will be continued with the view of establishing exacting criteria for sterilization of spacecraft and spacecraft components with dry heat and with ethylene oxide.

It is my opinion, in view of the obvious urgency of the space race, that long term research on other means of sterilization will not yield positive results within a time interval that can be considered useful.

While the question of the feasibility of achieving internal sterilization of all spacecraft components is obviously one of the most difficult tasks in the Bioscience Program, I assume that for the present this should be considered the aim. External component sterilization alone would seem to be acceptable only by great manipulation of hard landing probabilities.

The following R and D projects are based on the above line of thinking and are designed to support the sterilization guidelines developed at the ad hoc conference of July 9, 1962.

1. Processes and Materials for the Manufacture of Sterile Spacecraft Components.

The aims of this engineering study would be as follows:

a. To determine what materials and manufacturing processes now in use yield internally sterile components.

b. To determine what materials and manufacturing processes now in use yield components which will withstand subsequent heat sterilization.

c. To determine what feasible changes can be made in present manufacuring processes which will improve the probability of obtaining internally sterile components.

d. To determine what material substitutions can be allowed to improve the resistance of components to heat sterilization - during and after fabrication.

In this study, because of the thousands of spacecraft components involved, it will be necessary to prepare a reasonable type--classification of parts. Reduction to, say, approximately 100 component types will simplify the research approach.

2. Biomathematical Study of Sterilization Probabilities

This study by biomathematicians would be directed toward determining the parameters and statistical factors and limitations involved in the expression of spacecraft sterilization data in the terminology used in space-science for controlling trajectories. Defined methods for calculating biological probabilities should be developed in order to assure that data developed in the Bioscience Program can be easily and with reliance be used in all NASA undertakings. A part of this project would be the development acceptable definitions and criteria for establishing spacecraft sterilization probabilities.

3. A Study of Sterility Testing Methods and Standards

Based on presently available sterilization methods (principally dry heat and ethylene oxide), the purpose of this study would be to develop the best possible sterility test and monitoring methods in accordance with requirements established by spacecraft assembly and launching procedures. The prime evaluation criteria for candidate methods would be (1) accuracy of the method and (2) time required to obtain results. Since final culture tests for sterility at final assembly and launching would be only of historical interest, this study should consider reliable physical or chemical methods for predicting sterility.

4. Development of Improved Glove Boxes and Hoods and Glove Box Techniques

Since at the present time it is predictable that some spacecraft assemblies and/or repair operations will have to be carried out in externalization devices in which sterility is preserved or in which re-treatment sterilization is achieved, it is desirable to develop this isolation equipment to achieve maximum efficiency and convenience. This engineering research program should lead to the development of detailed design and construction criteria, based on accepted and tested prototypes, for the several types of isolation devices required. Because of the magnitude and variety of the manipulations on spacecraft equipment which will have to be carried out in

this equipment, the study should include a maximum effort in human factors engineering, and general operating procedures for performing the required tasks.

5. Psychological Assistance Research Study

This study by a competent group of psychologists, working with exobiologists and spacecraft engineers, would be directed primarily toward developing methods, techniques, and guidelines which would facilitate the incorporation of spacecraft sterilization procedures into the regular planetary flight programs. The study should be focused on the integration of the Control Sterility Group within the space program.

As pointed out by the participants at the Ad Hoc Sterilization Conference, human error probabilities are relatively large as compared to error probabilities not directly under the influence of human control. Influencing the successful operation of the Control Sterility Group, therefore, are many factors not directly covered by research in space science and exobiology. The probable impact of the control group on the regular NASA functions should be considered and ways sought to use psychological approaches to advantage wherever possible. For example, psychological evaluations could be made of the various levels at which responsibility for sterility could be assigned. Just how intergroup relations will effect the attitudes of NASA employees and decrease or increase the probability of sterilization failure can be studied. The specific approaches and interpersonal relations used by the Control Sterility Group could receive attention. Suitable attention should be given, also, to methods of instructing and training cognizant engineers.

6. Equipment and Methods Evaluation Study Group

It is suggested that a suitably equipped and housed equipment and methods group be organized to evaluate recommended procedures and equipment before they are passed to the Control Sterility Group for implementation. This will eliminate the burden of further development, research, or adaptions by the Control Sterility Group. The Equipment and Methods Evaluation Study Group would have as their primary mission the transition of laboratory-developed data and methods to practical on-the-site field use. Work with proto-types of field equipment is recommended. From this group, also, could come training devices and methods for use by the Control Sterility Group. The preparation of the suggested "Spacecraft Sterilization Handbook" would be accomplished by the equipment and methods evaluation group.

7. Spacecraft Sterilization Bibliography Services

It is recommended that, as a continuing service to the other R & D efforts, a small bibliography service be established. The function of this group would be to act as an assembly and clearing house for references, publications, reports, etc., developed by the Bioscience Programs, particularily information relative to spacecraft sterilization. By improving

the availability of research results between scientists in the several R & D projects much time and duplication of effort could be saved. This library group could also provide continuous screening of the literature for R & D information of probably interest to researchers in the Bioscience Programs.

Mr. A. M. Nowitsky

1. Sealed Cavity Research and Development

Methods for absolute sealing and efficient separation of sealed spacecraft compartments should be the subject of a research and development program. Considerations should include remote hermetic sealing, biological exhaust venting, efficient and reliable filtration and compatible design practices.

2. Internal Sterility Assurance

Whether or not an internally sterilizing heat load will be applied to spacecraft components after manufacture, the following research is recommended for added insurance: Although in many instances the manufacturing process indicates the possibility of a sterile end-product, it does not provide sufficient proof of internal sterility. Feasibility of altering the normal manufacturing process to provide sterile end-products should be studied. Development of process modifications, acceptable to the manufacturer, should be investigated. Sporicidal additives to sealants, lubricants, and other nonfunctional materials should be evaluated. Efficient aseptic subassemblies combined with compatible (modular) designs should be considered.

A study is recommended to investigate effects of electrical parameters normally applied to the component, on the test organism. It is possible that the combined effects of voltage, current, frequency, and temperature rise during normal operation might render components sterile. However, this procedure would be acceptable for sterilization only if performed before launch, because components malfunctioning in flight would not be sterilized.

3. Exobiological Sterilization

Prevention of possible mutual contamination by returning, contaminated probes remains an important consideration. Feasibility studies should be conducted to result in means for determining effect on earth-life cellular material, before any attempt at retrieval. Studies should be made concerning translation of this research data into practical hardware systems for remote exobiological sterilization, terminal with respect to earth reentry.

4. Sterilization Requirements for Manned Space Flight

Sterilization requirements for manned space flight should be thoroughly investigated and a number of research and development programs initiated. Areas for consideration include:

Spacecraft design, utilizing the maximum number of qualified sterile products with consideration of sterilization as an early design concept, including appropriate internal sterilization and aseptic assembly.

Internal sterilization of the unmanned spacecraft sections

Aseptic assembly of the internally sterilized sections

Aseptic launch, involving terminal surface sterilization of surface-contacting spacecraft elements

Sterile egress and ingress, accomplished through a specially designed entrance lock presterilized before launch, enabling rapid personnel transfer without mutual contamination

Safe waste disposal, to prevent putrefaction gas buildup, spacecraft living-environment air contamination, and biological seeding of extraterrestrial surfaces

Aseptic sampling, to prevent contamination of biological samples during the sampling operation

Aseptic retrieval, to protect biological sample results, and automatic terminal sterilization or destruction of sealed spacecraft and equipment not being returned to earth

5. Spacecraft Design Rules for Sterilization Compatibility

The design of extraterrestrial spacecraft should be compatible with sterilization techniques and physical parameter extremes. In particular, the following areas warrant thorough investigation:

Sealing, especially with respect to transfer locks, must yield biologically leaktight conditions

Hermetically sealed components, such as many electronic items, should be chosen from those known to be rendered sterile during normal manufacture

Maintainability, compatible with aseptic parts replacement, is desirable. Use of modular construction is stressed

Ground checkout and calibration receptacles should be designed to permit installation and removal of umbilical servicing lines without contamination

Venting of various exhausting media should be controlled to pass through biological filtration systems

Launch configuration should permit terminal sterilization of affected spacecraft structure and systems

6. Material and Component Compatibility/Reliability Research

An exhaustive program involving compatibility of all possible spacecraft materials and components with sterilizing heat, radiation, chemicals, etc., and subsequent reliability, is recommended. A rigidly enforced Qualified Products List should be evolved for industrial use. Materials research should be conducted in order to determine appropriate substitutes for materials currently being used in spacecraft manufacture but which are not compatible with nor adequately reliable after exposure to sterilization techniques. Sterilization processes must be considered as additional environmental stresses to be included in the normal stress and reliability testing programs required in spacecraft design and construction. Assessment of reliability degradation should be made. Analytical techniques and theoretical analysis for estimating reliability degradation as a function of temperature should be determined.

7. Basic Research

Studies are recommended to determine types and approximate number of microbes likely to exist on the outer and inner surface of large structural components, in the pockets formed in such components, screwholes, etc. Qualitative and semiquantitative analysis of types of contaminant should follow.

Efficacy of internal sterilization, involving effect of normal manufacture on the contamination level of sealed components as well as sterilization heat stabilization times, should be evaluated.

Special search for organisms to be used in establishing standard sterilization operating procedures should be conducted. Enrichment procedures, involving exposures to selected extremes (e.g., heat, radiation, and chemicals), carried out in selected media, should be utilized in determination of the most resistant strains.

With selected organisms used as "controlled contaminants," flight experiments should be conducted to permit the study of destruction by combinations of space vacuum, solar heat, solar ultraviolet, cosmic, and radiation belt irradiations.

Investigations should be made into the feasibility of avoidance of organic compounds in the building of spacecraft systems, especially protein-type materials such as wool and leather. Further studies should pertain to possible molecular marking of organic materials as being originated on

earth. In addition, ultimate removal of dead organic material due to the process of sterilization, should be explored.

Studies are recommended to determine the present state of organic chemical contamination in typical existing assemblies of propulsion stages.

8. Operations Research

A study program is recommended for determining world-wide seasonal bacteriological levels for earth atmosphere. Normal meteorological techniques should be utilized and combined with bacteriological instrumentation. Results of the experimental data obtained would be of value in establishing magnitude of the terminal sterilization, inflight sterility maintenance problems, and aseptic retrieval considerations.

NIH PROJECTS RELATED TO STERILIZATION

GRANT NO.	PROJECT	NAME, ADDRESS, PUBLICATIONS
E-2398C2	Sterilization and sanitization of bedding materials	Wedberg, S. E., U. Connecticut Storrs, Connecticut
E-3708	The destruction of bacteria by heat	Pflug, I. J., Michigan State U. East Lansing, Michigan
G-7564	Research on aseptic canning procedures	Leonard, S., Univ. California Davis, California
A-2904C4	Physiochemical basis of biological stability	Foster, J. F., Purdue Univ. West Lafeyette, Indiana
E-1863C3	Mechanism of heat resistance in bacterial spores	Sandoff, H. L., Michigan State U. East Lansing, Michigan
E-2896C1	Study of bacterial sporogenesis	Halvorson, H.O., U. Illinois Urbana, Illinois Biochem. Biophy. Res. Comm., v. 4, p. 409, Mar. '61
G-5315C3	Phosphate and sulfhydryl groups in proteins	Hill, R. M., Univ. Nebraska Lincoln, Nebraska
G-5808C2	Factors affecting the heat stability of proteins	Brown, D. K., Union College Lincoln, Nebraska
G-6110C2	Resistance of bacterial spores	Walker, H.W., Iowa State U. Ames, Iowa
E-2698C1	Nature of antibacterial action of beta-propiolactone	Teodoro, R. R., Wayne State U. Detroit, Michigan

Dr. E. Staten Wynne

1. Selection of microorganisms for controlled contamination procedure.

The development of adequate sterilization methods (dry heat, radiation, chemicals, etc.) necessitates the use of controlled contamination procedures with highly resistant microorganisms. More than one species of microorganisms will be required, because a species highly resistant to dry heat may not be relatively as resistant to radiation or chemicals. Research in the selection of microorganisms is necessary in order to assure the maximum confidence levels of sterility (99.99%).

2. Search for faster acting chemicals disinfectants, especially gaseous, for surface and/or terminal sterilization.

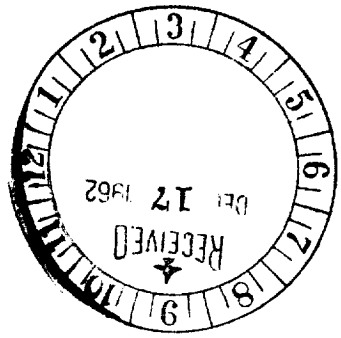
Current terminal sterilization with ethylene oxide requires approximately 16 hours. Faster acting chemical disinfectants would enhance count-down procedures, as well as reduce delays due to repairs, etc., on a previously sterilized vehicle.

3. The effects of sterilization procedures, especially dry heat and/or radiation on the performance characteristics, over long operating periods, of spacecraft components.

Many components, subjected to dry heat sterilization, may be damaged temporarily or even not at all by present test procedures. However, it is possible that temporary, or even currently undetectable, damage could reduce the effective life span performance sufficiently to result in malfunction. Components subjected to dry heat sterilization should be installed in circuits, and observations made to determine longevity and efficient performance.

4. Survival of earth microorganisms under simulated lunar and/or planetary conditions.

Specific knowledge of the atmospheric and surface composition of the moon and planets is required for determining survival of microorganisms under simulated conditions. As soon as data obtained with unmanned vehicle is available, survival of microorganisms should be determined. Such studies could enhance or reduce the sterilization requirements for the moon and/or the planets. For example, if earth life cannot survive and/or no life or pre-life forms are found on the moon, the sterilization requirements, especially for manned vehicles, will be reduced.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